SIMULTANEOUS REMOVAL OF ORGANIC MATTER AND NITRATE FROM AQUEOUS SOLUTIONS USING A BIO-ELECTROCHEMICAL REACTOR

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ABSTRACT

In this research an up-flow undivided bio-electrochemical reactor is used for treating simultaneously organic matter and nitrates in aqueous solutions. Nitrate elimination takes place at the cathode while organic substance is oxidized at the anode. The cathode material is palm shell granular activated carbon coated with a film of autohydrogenotrophic bacteria. A nano-crystalline PbO_2 coated carbon combination was compared against various anodic materials namely stainless steel, titanium, graphite and carbon felts. The results showed that PbO_2 provided better performance in the elimination of both pollutants. As such PbO_2 was used to study the effects of major operating parameters such as electrodes spacing, electric current and hydraulic retention time (HRT) on the performance of the reactor to treat organic matter and nitrates. Response surface methodology (RSM) was used to evaluate the interactions between these operating parameters and to optimize the reactor performance. The optimum conditions for the simultaneous elimination of organic matter and nitrate are an electrodes spacing of 3.2 cm, electric current of 18 mA and HRT of 45h that provided organic matter removal efficiency of 83% along with 99% elimination of nitrate. It was found that the current efficiency (CE) is unaffected by electrode spacing and is higher at low electric current and HRT. The control of pH is important to minimize nitrite-nitrogen accumulation.

ABSTRAK

Dalam kajian ini bio-reaktor electrokimia yang aliran atas dan tidak terbahagi telah digunakan untuk merawat serentak organik dan nitrat dalam berair. Penyingkiran nitrat berlaku serentak di katod manakala organik dioksidakan pada anod. Bahan katod adalah daripada kelapa sawit karbon aktif butiran yang disalut dengan lapisan bakteria autohydrogenotrophic. Komposit karbon bersalut PbO_2 nano-kristal telah dibandingkan dengan pelbagai bahan anodic seperti stainless steel, titanium, grafit dan karbon felts. Ia didapati bahawa PbO_2 memberikan prestasi unggul dalam penyinggiran kedua-dua bahan pencemar. Oleh itu, PbO_2 telah dipilihkan untuk menjalankan kajian tentang parameter operasi utama seperti jarak elektrod, bekalan elektrik dan hidraulik (HRT). Kaedah permukaan respons (RSM) adalah bertujuan untuk mengkaji interaksi semua parameter dan mengoptimumkan prestasi reaktor. Keadaan optimum untuk penyingkiran serentak bahan organik dan nitrat ialah jarak antara elektrod sebanyak 3.2 cm, arus elektrik sebanyak 18 mA dan HRT sebanyak 45 jam. Dalam keadaan optimum ini dapat memberikan kecekapan penyingkiran bahan organik sebanyak 83% dan penyingkiran 99% nitrat. Keberkesanan elektrik (CE) tidak dipengaruhi oleh jarak elektrod dan ia boleh dipertingkatkan dengan arus electric dan HRT yang rendah. Pengawalan pH adalah penting untuk mengelakkan pengumpulan nitrit-nitrogen..

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CHAPTER 1: INTRODUCTION

1.1 Introduction

Water is vital in life. The application of water includes agricultural, industrial, household, recreational and environmental activities. As a result of urbanization and increasing use of fertilizers that result in increasing toxic effluents in industrial wastewater, concerns have sprung up regarding the health consequences of the consumer. One of such concerns includes the increase of nitrate concentrations in groundwater. High nitrate concentration in drinking water causes methaemoglobinemia and gastric cancer. Methaemoglobinemia is also known as the blue baby syndrome and occurs normally in infants of ages 0-3 months because they have little methaemoglobin redustase. When nitrate enters human intestines, it is converted into nitrite and reacts with haemoglobin to form high amounts of methaemoglobin. Since methaemoglobin are non-oxygen carrying compounds, the infant's tissue and organs may lack oxygen that could result in death (Nitrates and nitrites, 2007). In order to protect consumers from the adverse effects of nitrates, the United States Environment Protection Agency (EPA) and World Health Organization (WHO) have set a maximum contaminant level (MCL) of 10mg NO_3^-–N/l in drinking water (Cast & Flora, 1998b).

Organic matter is the combination of carbon, hydrogen, oxygen and other trace elements. Some of organic compounds such as proteins, carbohydrates and fats are easily degraded by organisms. However, excessive amounts of degradable organics in water bodies are dangerous to aquatic life since organisms utilize dissolved oxygen to degrade the organic products. This results to competition towards dissolved oxygen between organisms and aquatic life and deteriorates the overall water quality (Mostofa et al., 2005; Nora'aini et al., 2005). Moreover, the organic compounds used in agriculture, textile and food industries are difficult to be degraded by organisms. This

kind of wastewater is hazardous and will contaminate the aquatic life and effects human being (OM., 2011). In addition, treatment costs will be increased when dissolved organic carbon in wastewater is higher (Mirzoyan et al., 2010). Hence, elimination of nitrate and organic matter from wastewater is an essential step before the wastewater is discharged to the environment.

The traditional methods to treat organic matter and nitrate are divided into physicochemical and biological treatments. The traditional physicochemical processes are successful in treating organic matter and nitrate but the treatments are expensive, they require regeneration and are unfriendly to the environmental (Feleke & Sakakibara, 2002; Lopez et al., 2004; Shawwa et al., 2001; Silva et al., 2004; Wan et al., 2009). The drawbacks of conventional biological treatment are excess sludge production and biogas generation which cause global warming (Hoilijoki et al., 2000; Nandy et al., 2002). Consequently, a new technology, bio-electrochemical technique is employed to treat these both pollutant compounds.

Bio-electrochemical method generally refers to the use of electric current passing through an electrode to enhance the degradation of contaminants by microorganism (Ghafari et al., 2009b). These microorganisms are normally adhered on to the electrode surface to exchange electrons (accept or donate) with solid electrodes to stimulate microbial metabolism (Nester et al., 2009). Bio-electrochemical systems can be applied for wastewater treatment and generation of renewable hydrogen gas. One of the most promising developments of bio-electrochemical technology is microbial electrolysis cell (MEC) in which organic compounds are oxidized by microorganisms at the anode producing carbon dioxide, protons and electrons. These electrons move to the cathode and reduce water molecules to hydrogen gas. Hence, bio-electrochemical system can be considered as green technologies since they eliminate organic compounds and generate hydrogen gas simultaneously from wastewater without

threatening the environment (Sleutels et al., 2009). This makes bio-electrochemical technology worthy of investigation on simultaneous organic matter degradation and denitrification. The technology involves denitrifying microorganisms immobilized on the cathode surface with the hydrogen gas as electron donor being produced at the cathode through water electrolysis; meanwhile organic matter is oxidized to carbon dioxide at the anode.

The electrode materials used for removing organic matter and nitrates in bio-electrochemical systems are platinum, stainless steel, titanium, PbO_2, carbon and graphite (Aboutalebi et al., 2011; Dumus et al., 2008; Prosnansky et al., 2002; Sakakibara & Nakayama, 2011; Tartakovsky et al., 2009; Zhou et al., 2009) . It is important to select a suitable anode material since the carbon dioxide generated from the anodic oxidation of organic compounds would change the pH by dissolving into water. Nitrate removal is highly dependent on the pH of the system. To date, there are is no studies on comparing different materials for the simultaneous removal of both pollutants.

It is essential to obtain the optimum operating parameters by response surface methodology (RSM). RSM is a useful tool to investigate the interaction of all parameters and optimize the performance of the process. In addition, RSM will produce a regression model equation which can be used in design purpose. Suitable ranges of the operating parameters should be determined prior to the RSM study.

1.2 Objectives

The main objectives of this research are

i. To study the effect of different anode electrode materials on the removal of organic matter and nitrate-nitrogen in a continuous bio-electrochemical reactor.

ii. To determine the range of operating parameters such as electrode spacing, electric current and hydraulic retention time (HRT).

iii. To determine the optimum operating conditions for organic matter and nitrate removal by response surface methodology.

1.3 Outline of thesis

Chapter 1: Introduction

This part encompasses the introduction of the research background, objectives and outline of this thesis.

Chapter 2: Literature review

This part depicts each single phase of this study. This encompasses a review on traditional method, electrochemical and bio-electrochemical technology which applied to deal with organic matter and nitrate. A brief assessment on operating parameters in bio-electrochemical process is added as well.

Chapter 3: Methodology

This part demonstrated descriptive information about instruments and experimental techniques. Analytical procedures for determination concentration of organic matter and nitrate are presented in this part

Chapter 4: Results and Discussion

This part contains the experimental outcomes with a detail discussion.

Chapter 5: Conclusion and recommendation

The final part of this paper concludes all facts and findings in this study and recommends some potential suggestions for further investigation.

CHAPTER 2: LITERATURE REVIEW

2.1 Conventional treatments

The conventional techniques to remove organic matter and nitrate are divided into two main categories: physicochemical and biological. Physicochemical treatment methods include coagulation, flocculation, adsorption, oxidation, membrane treatment, ion exchange (IE) and reverse osmosis (RO); while biological treatments are aerated and anaerobic lagoons, activated sludge process, upflow anaerobic sludge blanket (UASB), anaerobic filter, anaerobic lagoon and fluidized bed reactor. All the conventional treatments have their advantages and disadvantages which are briefly reviewed below.

2.1.1 Conventional organic matter removal methods

The physicochemical treatments to remove organic compounds are coagulation, flocculation, adsorption, oxidation and membrane treatment. Aluminium sulfate, ferrous sulfate, ferric chloride and ferric chloro-sulfate are normally used as coagulants but it produces sludge and residue aluminum or iron in the end of experiment (Dilek & Gokcay, 1994.; Silva et al., 2004). Activated carbon is widely utilized as adsorbent for removing organic matter but it required regeneration activated carbon frequently and its higher porosity could become a breeding ground for microorganisms (Aloui et al., 2009; Shawwa et al., 2001). The typical chemical oxidation process is combination of strong oxidants, e.g. O_3/H_2O_2, irradiation, e.g. ultraviolet/ultrasound, catalysts, e.g. transition metal ions/photo catalysts which successfully remove organic matter (Huang et al., 1993; Kulkarni, 1998). However, the drawbacks of chemical oxidation process are the intermediate oxidation reaction. The products are toxicity and treatment costly with consumption of electrical energy for devices such as ozonizers, UV lamps, ultrasounds are higher (Lopez et al., 2004). Although membrane treatment is efficient in reducing

organic compounds in most of the cases, the big challenges of this process have to face is fouling problem (Sakinah et al., 2007).

Biological treatment is commonly used for the removal of organic matter due to good reliability and effective. Organic compounds are degraded to carbon dioxide and sludge under aerobic environments and to biogas under anaerobic conditions (Nester et al., 2009). Aerobic treatment is included aerated lagoons, activated sludge process and aerobic biological reactors. Aerated lagoons are successful remove COD over 95% and this method is popular employed since their low in operation and maintenance cost (Maynard et al., 1999; Rodriguez lglesias et al., 2000). Activated sludge process consequent excess sludge production and require high energy processing as well as longer aeration times (Hoilijoki et al., 2000; Loukidou & Zouboulis, 2001). Up-flow anaerobic sludge blanket (UASB), anaerobic filter, fluidized bed, anaerobic lagoon and anaerobic contact reactors are anaerobic treatments that are commonly used to treat high organic loading rates and biogas generated as the final product (Nandy et al., 2002). UASB process has high organic removal efficiency and short hydraulic retention time but it easier inhibited by toxic compounds (Renou et al., 2008). The overall advantages of anaerobic treatment are low energy cost and less sludge production but they are limited in removal pathogens and has serious odor problem (Satyawali & Balakrishnan, 2008).

2.1.2 Conventional nitrate removal methods

Physicochemical methods for nitrate removed include ion exchange (IE) and reverse osmosis (RO). The advantages of using RO include high permeability efficiency of selective ions, low production costs, environmental friendly consequences, unchanged molecular structure in separation process at room temperature and no product accumulation in the membrane (Lopez-Ramirez et al., 2006). The limitation of RO technique is that the wastewater requires further treatment as the nitrate removed is

accumulated in the brine system (Matos et al., 2009). Efficiency of permeability becomes limited when soluble salts such as calcium carbonate (CaCO3) and calcium sulphate (CaSO4) from the feed solution, precipitates on the membrane (Hasson et al., 2001). Fouling problem is also an issue as it affects the membrane performance and increases complexity in the membrane operations.

Ion exchange resins are initially bonded to functional groups in chloride ions. The chloride ions are exchanged with anions and flow out from the system when contaminated water passes through the resin beads. The resin beads can be regenerated with sodium chloride solution by displacing the anions by chloride ions. However, this is not always a straightforward task when the anions have more affinity to the resin than the chloride ions (Roquebert et al., 2000; Velizarov et al., 2008; Xu et al., 2011). This contributes to higher operation cost since extra steps have to be taken to eliminate the anions before being discharged to the environment (Shrimali & Singh, 2001).

Biological treatments are carried out by bacteria that convert nitrate to nitrogen gas. Trickling filters consist of a fixed media bed through which prefiltered wastewater trickles downwards over an aerobic biofilm (Lekang & Kleppe, 2000). Although high nitrate removal rate is observed in trickling filters, biofilm shedding and high risk of clogging during operation caused the imperfect of nitrate elimination (Eding et al., 2006). Fluidized bed reactor is one of the solutions for clogging problems in trickling filters but its overall treatment cost is higher than other since it required additional aeration system to launch the treatment (Summerfelt, 2006).

2.2 Electrochemical technology

The conventional methods do help with organic matter and nitrate removal but the disadvantages include sludge production, high energy demand, unstable performance and frequent maintenance requirements. Hence, research on new methods

for nitrate and organic matter removal in wastewater is under way. The past few decades has seen the emergence of electrochemical technology for wastewater treatment. The particular advantages of electrochemical treatment include high efficiency, ambient operating conditions, small equipment sizes, minimal sludge generation and rapid start-up (Dash & Chaudhari, 2005; Grimm et al., 1998; Li et al., 2009a).

2.2.1 Electrochemical oxidation of organic matter

Electrochemical oxidation of organic matter can take place directly on the anode surface or indirectly in the bulk of electrolyte. Organic compounds can be oxidized directly at anode surfaces through physically adsorbed hydroxyl radicals, $MO_x(^{\bullet}OH)$ that carbon dioxide is the final product. This hydroxyl radical also produces higher oxide species (MO_{x+1}) on dimensionally stable anodes (DSA). DSA is an inert metal coated with noble metal oxides such as RuO_2 and IrO_2. The mechanism of direct anodic oxidation is illustrated in Table 2.1.

For direct anodic oxidation, the electrode material is the main figure of merit. Many researchers found their particular interests on boron doped diamond (BDD) and metal oxide anodes. Under the same operating condition, BDD showed much superior organic matter removal efficiency as compared to Ti/SnO_2, Ti/IrO_2, $Ti\text{-}Ru\text{-}SnO_2$ and PbO_2 (Panizza & Cerisola, 2007; Waterston et al., 2006). This is due to BDD has higher potential to produce hydroxyl radicals compared with metal oxide, but BDD anode is extremely high cost (Martinez-Huitle & Ferro, 2006). Oxygen evolution reaction is an undesirable side reaction and considered as a factor that limits the efficiency of the electrochemical process (Comninellis, 1994; Martinez-Huitle & Ferro, 2006). Therefore, oxidizing agents are recommended to be used to increase the oxidation rate.

Table 2.1: General mechanism of direct anodic oxidation of organic compounds

Process	Reaction steps
Water is electrolyzed by metal oxide to produce adsorbed hydroxyl radicals	$MO_x + H_2O \rightarrow MO_x(^{\bullet}OH) + H^+ + e^-$
Oxidation of organic compounds by $MO_x(^{\bullet}OH)$	$R + MO_x(^{\bullet}OH) \rightarrow CO_2 +$ inorganic ions $+ MO_x + H^+ + e^-$
Formation of higher oxide species	$MO_x(^{\bullet}OH) \rightarrow MO_{x+1} + H^+ + e^-$
Oxidation of organic compounds by higher oxide	$MO_{x+1} + R \rightarrow MO_x + RO$
Oxygen evolution via adsorbed hydroxyl radicals	$MO_x(^{\bullet}OH) \rightarrow MO_x + ½\ O_2 + H^+ + e^-$
Oxygen evolution through higher oxide	$MO_{x+1} \rightarrow ½\ O_2 + MO_x$

Indirect electro-oxidation is achieved through the use of oxidizing agents such as peroxide, Fenton's reagent, sodium chloride, chlorine, hypochlorite or peroxodisulfate. Oxide electrodes are very active for Cl_2 evolution, so this agent is commonly used in oxidation of organic matter (Bonfatti et al., 2000; Feng et al., 2003; Martinez-Huitle & Ferro, 2006). Chloride oxidizes to form chlorine (Cl_2) and further reacts with water to produce hypochlorous acid (HOCl) and hypochlorite ions (OCl^-). Then, hypochlorite ions oxidize the organic matter to produce carbon dioxide. The major reaction mechanisms of indirect anodic oxidation are given in Table 2.2. However, indirect oxidation of organic matter will form intermediates such as organochlorine, perchlorate compounds which are the factor of mutagenic and carcinogenic (Bergmann & Rollin, 2007; Chen, 2004).

Another type of oxidation organic matter is electro-Fenton method. Fenton reagent is used to produce OH radicals by addition of hydrogen peroxide to Fe^{2+} salts. The Fe^{2+} can be regenerated by reducing the ferric ion (Fe^{3+}) (Wang et al., 2010; Zhang et al., 2006). Electro-Fenton process is more economical and efficient in removing organic matter compared to the conventional Fenton process since it using electrochemical technology to generate hydrogen peroxide at cathodic side (Umar et al.,

2010). In electro-Fenton process, removal of organic matter is directly proportional to the concentration of oxidizing agent used, but an excess of peroxide will be found in the end of the treatment (Konstantinou & Albanis, 2004; Virkutyte & Jegatheesan, 2009).

Table 2.2: General mechanism of indirect anodic oxidation of organic matter

Oxidizing agent	Reaction steps
Chloride	$2Cl^- \leftrightarrow Cl_2 + 2e^-$
	$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$
	$HOCl \leftrightarrow H^+ + OCl^-$
	$OCl^- + R \rightarrow Intermediates \rightarrow CO_2 + Cl^- + H_2O$

Table 2.3: Formation of reactive hydroxyl radicals in electro-Fenton process

Oxidizing agent	Reaction steps
Fenton reagent	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$
	$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+$

2.2.2 Electrochemical reduction of nitrate

Electrochemical technology can be applied to reduce nitrate ions to nitrite and finally to nitrogen gas on the cathode surface. Nitrate (NO_3^-) and nitrite ions (NO_2^-) are very soluble in water and form several types of products. Nitrite ions act as intermediate products and further react with water to generate nitrogen gas, ammonia and hydroxylamine (NH_2OH). Reduction of nitrate to nitrogen gas is the desired process but ammonia is usually formed. Sodium bicarbonate ($NaHCO_3$) is required to maintain the pH during electrochemical reduction of nitrate since the electrolyte gradually becomes alkaline (Li et al., 2009c; Paidar et al., 2002). High alkaline environments prompt the generation of precipitates of magnesium hydroxide and calcium carbonate around the cathode when soluble calcium and magnesium salts are present in the water (Hasson et al., 2010). Ammonia and nitrite are the two main end products generated and are considered as major limitations to the efficacy of electrochemical denitrification.

Chloride-salt is widely added to overcome this issue. In this process, chlorine is oxidized at the anode and reacts with water to form hypochlorous acid (HOCl). The hypochlorite ions then react with nitrite and ammonia to produce nitrate and nitrogen. The general mechanism of the electrochemical reduction of nitrate has been summarized in Table 2.4. (Abuzaid et al., 1999; Chakrabarti et al., 2011; Li et al., 2009a; Li et al., 2009b; Paidar et al., 2002).

Table 2.4: General mechanism involved in the electrochemical reduction of nitrate

Process	Reaction steps
Cathodic water electrolysis	$2H_2O + 2e^- \rightarrow H_2 + 2OH^-$
Anodic water electrolysis	$4OH^- \rightarrow O_2 + 2H_2O + 4e^-$
Reactions of nitrate ion and water molecules	$NO_3^- + H_2O + 2e^- \rightarrow NO_2^- + 2OH^-$
	$NO_3^- + 3H_2O + 5e^- \rightarrow ½N_2 + 6OH^-$
	$NO_3^- + 6H_2O + 8e^- \rightarrow NH_3 + 9OH^-$
Reaction of nitrite ion and water molecules	$NO_2^- + 2H_2O + 3e^- \rightarrow ½N_2 + 4OH^-$
	$NO_2^- + 5H_2O + 6e^- \rightarrow NH_3 + 7OH^-$
	$NO_2^- + 4H_2O + 4e^- \rightarrow NH_2OH + 5OH^-$
Reduction of nitrate (especially sodium nitrate) to produce ammonia	$NO_3^- + 2H_2O \rightarrow NH_3 + 2O_2 + OH^-$
Sodium bicarbonate added to maintain pH of electrolyte	$NaNO_3 + NaHCO_3 + H_2O \rightarrow NH_3 + 2O_2 + Na_2CO_3$
Chlorine formed in anodic electrolysis	$2Cl^- \rightarrow Cl_2 + 2e^-$
Reaction of chlorine and water molecules	$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^- +$
Reaction of nitrite and hypochlorite ions	$NO_2^- + HOCl \rightarrow NO_3^- + Cl^- + H_2O$
Reaction of ammonium and hypochlorite	$2NH_4^+ + 3HOCl \rightarrow N_2 + 5H^+ + 3Cl^- + 3H_2O$

Dash et al. (2005) had proved that metal cathode gave better nitrate removal than non-metal such as graphite. Anode material is also the factor on electrochemical denitfication which showed by Li et al. (2009a), used different types of materials as anodes and fix the cathode material to perform nitrate reduction. There is no certain pH

value for electrochemical denitrification because it is depends on other parameters such as cell configuration, electrode materials and applied current.

2.3 Bio-electrochemical technology

Bio-electrochemical system (BES) is using electric current passes through an electrode to enhance biological contaminant degradation (Ghafari et al., 2009b). The microorganisms is normally adheres on to the electrode surface to exchange electrons (accept or donate) with solid electrodes to stimulate microbial metabolism called as bio-electrode (Nester et al., 2009). BES can be applied for wastewater treatment and generation of renewable hydrogen gas (Marcus et al., 2011; Pham et al., 2009). BES can be classified as green technology since it converts organic waste to chemical energy without threatening the environment (Sleutels et al., 2009). For using hydrogenotrophic denitrifiers to reduce nitrate to nitrogen gas, external electrical energy is required to produce hydrogen since these microorganisms are utilizing hydrogen as energy source.

2.3.1 Bio-electrode

Bio-electrode has been divided into two categories which are bioanode and biocathode. In anodic chamber, the microorganism uses organic substrate as carbon sources and electron donors to produce energy carrier molecule (ATP). The organic substrates are converted through glycolysis and then processes into citric acid cycle to oxidize which will release carbon dioxide molecules. Meanwhile, NAD^+ (nicotinamide adenine dinucleotide) and FAD (flavin adenine dinucleotida) are reducing to their electron carrier forms, NADH and $FADH_2$ as shown in Figure 2.1. These electron carriers transfer their electrons from cytoplasm (citric acid cycle location) to membrane cell and then shuttled to anode through direct or mediated electron transfer mechanism (Nester et al., 2009; Watanabe, 2008). In other words, anode played a role as external electron acceptor for oxidation of organic substrates.

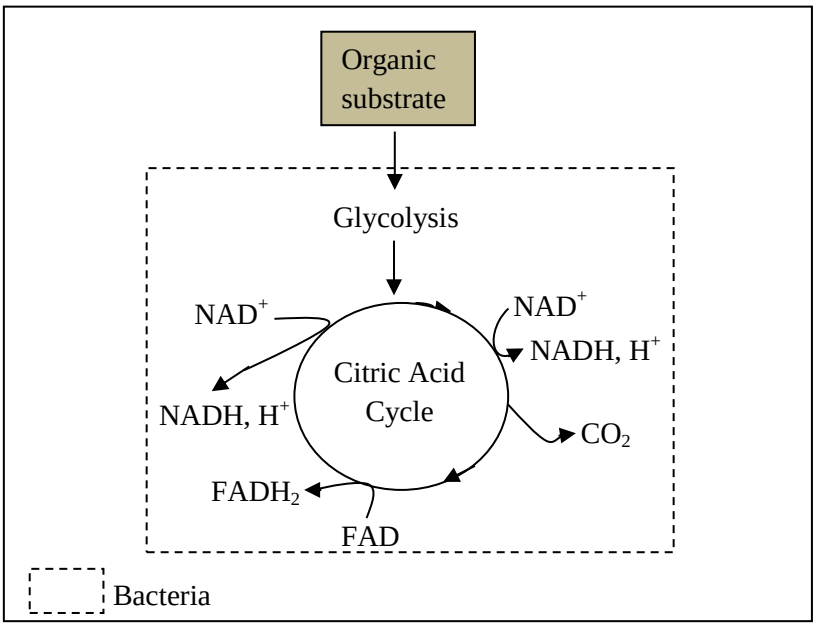


Figure 2.1: Reduction of NAD^+ and FAD through citric acid cycle (Schaetzle et al., 2008)

For biocathode, bacteria are used as biocatalyst to accept electrons from the electrode to replace the use of costly chemical catalysts (Lefebvre et al., 2008; Puig et al., 2011). The electrons should pass to high electro-positive electron acceptors such as oxygen, nitrate and chlorinated organic compounds by outer membrane cytochromes. Thus, standard oxidation-reduction potential (E^0) will be higher depicted that electrons are easier take up by microorganisms (He & Angenent, 2006; Huang et al., 2011b; Rosenbaum et al., 2011).

2.3.2 Electron transfer mechanism

Electrons transfer by microorganism can be categorized to two main groups, direct and mediated electron transfer which showed in Figure 2.2. Direct electron transfer (DET) is referred to a direct contact between bacterial active centre cell membrane enzyme (inner membrane, periplasmic and c-type cytochrome) and electrode surface (Huang et al., 2011b; Rozendal et al., 2008). These microorganisms should have membrane bound electron transport protein relay the electrons transferred from inside of bacterial cell to its outside (electrode) or vice versa (Schröder, 2007). Schaetzle et al. (2008) had summarized that the DET rate is very low due to the active size of the

enzyme is inside the protein environment. Some of the exoelectrogens species' redox enzymes are located at the outer surface of microorganism membrane, so the active sides of the redox enzymes are directly facing towards the electrodes or medium. However, this DET method required physical contact between bacterial cell, cytochrome and electrode. Hence, only bacteria in the first monolayer at electrode are electrochemically active. The bacteria, which is utilized DET in anodic and cathodic reactions come under the families of *Shewanella*, *Rhodoferax* and *Geobacter* (Aldrovandi et al., 2009; Du et al., 2007; Huang et al., 2011b; Watanabe, 2008).

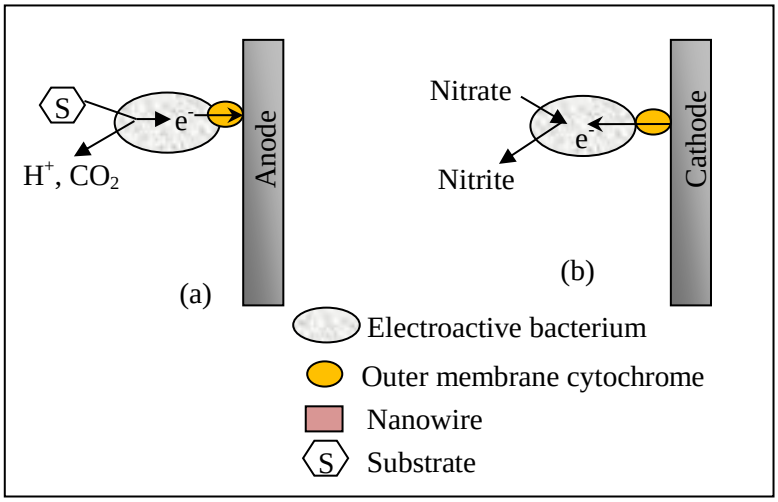


Figure 2.2: Direct electron transfer mechanisms in bio-electrodes. (a) Bio-anodes (b) Bio-cathodes (Pham et al., 2009; Rosenbaum et al., 2011)

Majority of microorganisms are unable to carry electrons directly to the electrodes since their outer layers consist of the non-conductive lipid membranes, peptididoglycans and lipopolysaccharides. Hence, mediators are required to shuttle electrons between electrodes and microorganisms (Du et al., 2007; Rosenbaum et al., 2011). The common exogenous synthetic mediators are methyl viologen, anthraquinone-2,6-disulfonate (AQDS), neutral red, humic acids and sulphur (Lojou et al., 2002; Park et al., 1999; Thrash et al., 2007). These redox mediators are not consumed by microorganisms and will recover at electrode. Nevertheless, using redox mediator to stimulate the electron transfer in BER is environmental unfriendly and

endanger healthy. Another type of MET is not require artificial redox mediator and it will generate themselves. Some microorganisms are able to synthetic redox mediators through primary and secondary metabolites consequent that electron transfer is independent on exogenous redox shuttles (Pham et al., 2009). The mediator acted as reversible electron acceptor or donor, transferring electrons either from bacterial cell to anode or from cathode to bacterial cell. However, now only phenazines (redox mediator) generated by Pseudomonas aeruginosa has been used in electron transfer between bacteria and anode (Rabaey et al., 2005).

2.3.3 Factors controlling organic matter removal

Biofilm on the anode surface will hydrolyze complex organic matter into simple molecules before being oxidized electrochemically by active microbes (Jiang et al., 2010; Marcus et al., 2011). Food industry effluents have been successfully treated by BER since their organic matter is easier be oxidized by microorganism (Cercado-Quezada et al., 2011). The characteristics of microorganisms used in BES are capability to hydrolyze cellulose, good electrochemical activity and use anode as an electron acceptor when oxidizing metabolites of cellulose hydrolysis (Pant et al., 2010). There are few main parameters that play important roles in bio-anode organic removal.

2.3.3.1 Anodic material and surface area

Although platinum anodes are successful in removing organic matter contaminants, the process can be very expensive. Stainless steel is one of the popular anodic materials and gives good results in eliminating organic species, but carbon based materials such as carbon and graphite are the most promising materials because of their stability when microbial cultures are grown on them and can ensure cheaper process costs (Dumas et al., 2008; Jadhav & Ghangrekar, 2009). Pre-treatment of carbon type electrode by ammonia and oxidation in sulphuric acid or nitric acid is the essential step

to improve the biofilm microbial composition and electron transfer because it would generate carboxyl functional groups (Cercado-Quezada et al., 2011). The bacteria which are able to catalyze the oxidation of organic matter and transfer electrons directly are easily colonized on graphite electrode surfaces and can transfer electrons to the anode with relative ease (Bond et al., 2002; Chaudhuri & Lovley, 2003; Kim et al., 2002; Tender et al., 2002). However, their ohmic resistance is 1000 times higher than metals (Pandit et al., 2011). Some researchers have modified graphite electrodes by coating with electron mediators, active polymers, polyaniline and quinone groups to improve their performance (Huang et al., 2011a; Sar et al., 2005; Wang et al., 2009).

Larger surface areas provide more space for microbial attachment which results in increased electron transfer rates. Some researchers have utilized graphite or carbon in different forms such as granules, felt, foam, nanotubes, fibers and others to increase the anodic surface area (Biffinger et al., 2007; Catal et al., 2008; Zou et al., 2008).

2.3.3.2 pH of the electrolyte

pH is a crucial parameter to control the activity of anodic respiration bacteria (ARB) (Nimje et al., 2011). The optimum pH for microbial activity is between 6.0 and 7.0. Organic matter removal at pH higher than 7.0 is actually the organic matter used in methanogenesis processes which form methane by microbial action and results in global warming (Jadhav & Ghangrekar, 2009; Sar et al., 2005). Although the anode plays a role in accepting electrons, it only transfers electrons to the cathode for completing the circuit and without changing the oxidation state of the electro-active species. Hence, anodic organic matter oxidation generates excess of H^+ ions which in turn lower pH at the anodic chamber. This would reduce the ARB performance since its optimum activity is in neutral conditions (Li et al., 2011; More & Ghangrekar, 2010).

To overcome this issue, a base buffer (normally a phosphate buffer) or carbonate has to be added to combine with H^+ and then form a weaker acid. Equation 2.1 to 2.3 indicate acid-base buffer and acid-carbonate buffer equilibrium reactions where Alk^- is alkalinity, HAlk is protonated alkalinity, CO_3^{2-} are carbonate ions, HCO_3^- are bicarbonate ions, H_2CO_3 is carbonic acid and CO_2 is carbon dioxide gas (Oh et al., 2010; Santoro et al., 2011; Tsan et al., 2011). Carbonate buffer is more beneficial than phosphate buffer in adjusting pH since inorganic carbon is available in all natural water (Marcus et al., 2011). Besides that, carbonate and bicarbonate can be reused in controlling pH since carbon dioxide gas generated from acid-carbonated buffer reactions (Eq. 2.4) is recycled internally to the cathode and then re-produce carbonate and bicarbonate again. The carbonate and bicarbonate ions are diffused back to the anodic chamber and the recycle process is repeated again (Tsan et al., 2011). The results of organic matter removal had been summarized in Table 2.5.

$$Alk^- + H^+ \leftrightarrow HAlk \quad (2.1)$$

$$CO_3^{2-} + H^+ \leftrightarrow HCO_3^- \quad (2.2)$$

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \quad (2.3)$$

$$H_2CO_3 \leftrightarrow CO_2 + H_2O \quad (2.4)$$

Table 2.5: A summary of results obtained by various workers in BES organic matter removal

Cell configuration	Electrode material		Experimental Conditions	Results	References
	Anode	Cathode			
Divided electrolysis cell with proton exchange membrane	Graphite granules and graphite rod	Graphite rod	pH 7 at anodic and cathodic compartment. Reactor 1: 6130 mgVSS/L Reactor 2: 4550 mgVSS/L Reactor 3: 3360 mgVSS/L	<u>Reactor 1</u> COD removed: 40±2.0 ppm/h Ammonia removed: 1.37 ppm/h <u>Reactor 2</u> COD removed: 25±1.3 ppm/h Ammonia removed: 0.54 ppm/h <u>Reactor 3</u> COD removed: 24±1.2 ppm/h Ammonia removed: 0.53 ppm/h	Aboutalebi et al., 2011
Electrolysis cell divided with 3mm J-cloth and either Nafion 117 membrane presented	5mm thick carbon felt	Gas diffusion electrode with a Pt load of 0.5 mg/cm^2	Acetate: 4.4 $gL_A^{-1}d^{-1}$ Continuous mode pH 7 Ww flowrate: 5 ml/d Trace metal solution flowrate: 146 ml/d HRT: 24 hr 1.0 V	<u>With Membrane</u> H_2 production: 5.57 L_{STP} $L_A^{-1}d^{-1}$ Acetate removal: ~98% <u>Without Membrane</u> H_2 production: 1.22 L_{STP} $L_A^{-1}d^{-1}$ Acetate removal: ~42%	Tartakovsky et al., 2009

Table 2.5: A summary of results obtained by various workers in BES organic matter removal (Continued)

Cell configuration	Electrode material		Experimental Conditions	Results	References
	Anode	Cathode			
Electrolysis cell divided with 0.7 mm polyester cloth	5mm thick carbon felt	Carbon paper gas diffusion electrodeposited with Ni	Applied voltage: 1.09-1.2V Standard phase OLR: 4 g $L_R^{-1}d^{-1}$ HRT: 9.5 h Influent: 1.6 g/L Phase 1: Varying OLR OLR: 8 g $L_R^{-1}d^{-1}$ HRT: 9.5 h Influent: 3.3 g/L Phase 2: Varying HRT OLR: 4 g $L_R^{-1}d^{-1}$ HRT: 16.5 h Influent: 3.1 g/L	Standard phase COD removal: 85% H_2 generated: 79% CE: 79.1% Phase 1 COD removal: 92.5% H_2 generated: 86% CE: 69.8% Phase 2 COD removal: ~100% H_2 generated: 79% CE: 81.5%	Tartakovsky et al., 2011

Table 2.5: A summary of results obtained by various workers in BES organic matter removal (Continued)

Cell configuration	Electrode material		Experimental Conditions	Results	References
	Anode	Cathode			
Single chamber mediatorless	Graphite brushes ($0.22m^2$)	Wet-proofed carbon cloth ($7cm^2$) with platinum catalyst	Fed batch mode Set 1 (Varying OLR) Influent: 1,2 and 3 g/L Applied voltage: 0.5V Set 2 (Varying applied voltage) Influent: 1 g/L Applied voltage:0.5 and 0.9V	Set 1 (1 g/L) CE: 99 ± 10% H_2 generated: 80% Set 1 (2 g/L) CE: 43 ± 1% H_2 generated: 31 ± 1% Set 2 (0.5V) CE: 84 ± 11% H_2 generated: 82 ± 5% Set 2 (0.9V) CE: 91 ± 10% H_2 generated: 87 ± 4%	Selembo et al., 2009

Table 2.5: A summary of results obtained by various workers in BES organic matter removal (Continued)

Combination dark fermentation and BES	Graphite fiber brush	Flat carbon cloth with Pt catalyst	BES Applied voltage: 0.5V Acclimated with FEI and SSI	FEI COD removal: 89±5% Hydrogen yield: 800±290 mL H_2/g-COD SSI COD removal: 91±2% Hydrogen yield: 980±110 mL H_2/g-COD	Lalaurette et al., 2009

ww: wastewater

SDR: Substrate degradation rate (kg COD_R/m^3-day)

SPY: specific power yield (W/Kg COD_R)

CE: coulombic efficiency where substrate degraded to produce electrons

OLR: organic loading rate

FEI: Fermentation effluent inoculums

SSI: Single substrate inoculum

2.3.4 Factors controlling denitrification

Autotrophic denitrification gives more advances in removal efficiency compared to heterotrophic denitrification since no further process required removing excess substrate and biomass production. Hydrogen gas is generally chosen as an electron donor source in autotrophic denitrification process since it has lower cost and does not generate any toxic byproducts (Sunger & Bose, 2009). However, hydrogen gas has low solubility in water and is also easier explosive (Szekeres et al., 2001; Virdis et al., 2010). Hence, some researchers had investigated a method to immobilize denitrifying bacteria on the cathode surface and utilized hydrogen gas produced from the electrolysis of water (Prosnansky et al., 2002). The denitrification reactions utilize hydrogen produced from the cathodic water electrolysis are depicted in Equation 2.5 to 2.8 (Ghafari et al., 2009b). There are general factors that affects on denitrification in BES includes electrode material, pH and electric current.

$$2H_2O + 2e \rightarrow H_2 + 2OH^- \quad (2.5)$$

$$NO_3^- + H_2 \rightarrow NO_2^- + H_2O \quad (2.6)$$

$$2NO_2^- + 3H_2 \rightarrow N_2 + 2H_2O + 2\ OH^- \quad (2.7)$$

Overall reaction:

$$2NO_3^- + 5H_2 \rightarrow N_2 + 4H_2O + 2OH^- \quad (2.8)$$

2.3.4.1 Cathodic material

Carbon material has sufficient mechanical strength and a rough surface which is ideal for the formation of biofilm as compared with stainless steel (Biffinger et al., 2007; Dumas et al., 2008). However, carbon material is difficult to apply in large scale processes due to its brittleness, bulky nature and high electrical resistivity caused larger electrode ohmic losses. Hence, graphite and carbon electrodes are supported by a conductive metal current collector such as stainless steel mesh (Rozendal et al., 2008).

Some researchers had suggested stainless steel and platinum more suitable used as cathodic material due to their strength, common manageability and better electrokinetic properties to support biofilm driven reductions as compared with carbon materials (Cast & Flora, 1998a). But, there is easier formation of a platinum oxide (PtO) layer at platinum electrode surface which will disrupt the denitrification process (Du et al., 2007). Graphite granules have been widely used in treating wastewater due to its large surface area that allow more bacteria attached on it and acted as third bipolar electrode (Zhou et al., 2009).

2.3.4.2 pH of the electrolyte

The pH of the wastewater is one of factor affect the performance of hydrogenotrophic denitrification. Nitrite concentration is higher when the pH value is more than 8.6 whereas when pH less than 7.0, carbonate ions are decomposed and hence decreases the nitrate removal rate (Karanasios et al., 2010). During batch denitrification process, pH of the solution increases and is normally adjusted between 6.5 and 7.0 by using phosphoric acid (Cast & Flora, 1998a). pH can also be adjusted by carbon dioxide gas produced at the anode which is controlled by electric current (Sukkasem et al., 2008). Equation 2.9 to 2.11 indicates the mechanism of adjusting pH by carbon dioxide where carbon dioxide dissolves in water to form carbonic acid then reacts with OH^- to produce HCO_3^-. HCO_3^- further reacts with OH^- to form CO_3^{2-}. This mechanism shows that using carbon dioxide can increase electric conductivity in water and lower ohmic potential drop since more ions (HCO_3^-, CO_3^{2-}) are present in the electrolyte (Prosnansky et al., 2002). Clauwaert and his co-workers (2009) had proved that only 26% of nitrate had been removed without pH adjustment, whereas the nitrate removal increased when the pH maintained at neutrality. The optimal pH for denitrification is between 6.5 and 8.0 which agreed with few researchers.

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (2.9)$$

$$H_2CO_3 + OH^- \rightarrow H_2O + HCO_3^- \quad (2.10)$$

$$HCO_3^- + OH^- \rightarrow H_2O + CO_3^{2-} \quad (2.11)$$

2.3.4.3 Electric current

Electric current would influence the hydrogen formation at the cathodic chamber, indirectly, it play a vital role in nitrate reduction since hydrogen is required for autotrophic denitrification. Some researchers are proved that bio-cathode denitrification is more advanced at lower electrical currents due to hydrogen is the limiting factor in the process (Cast & Flora, 1998b; Zhang et al., 2005). When electric current is higher, hydrogen gas production through electrolysis is increased and effervescence can be clearly observed. The gas bubbles causes channeling in granular activated carbon beds and forms a dry space which lowers the denitrification performance (Szekeres et al., 2001). Besides that, higher electric current enhances the production of oxygen at the anodic side which would compete with hydrogen generation to lower the hydrogenotrophic denitrification reaction (Wan et al., 2009). The applied current trend also supported by Flora et al., (1994) who concluded that larger current density yield excessive hydrogen gas which remains in biofilm and caused decreases denitrification rate (Flora et al., 1994).

The bio-cathode denitrification technique has been applied by many researchers to remove nitrate and a summary of their results are presented in Table 2.6.

Table 2.6: A summary of results obtained by various workers in bio-cathode denitrification

Cell configuration	Electrode material		Experimental Conditions	Results	References
	Anode	Cathode			
Anode is surrounded by biofilm cathode and combination with adsorption column	Carbon (160 cm^2)	251cm^2	Continuous mode HRT: 10 hr Electric current: 5 mA Initial nitrate: 22.5 mg/L	NO^-_3-N removal: 54.67% NO^-_2-N: 0.01 mg/L	Feleke & Sakakibara, 2002
Divided electrolysis cell with cationexchange membrane	Stainless steel	Stainless steel wrapped with stainless steel mesh	pH 7 Electric current: 1 mA Initial nitrate: 20 mg NO^-_3/L	Without heavy metal Current efficiency: 54.3% With heavy metal Current efficiency: 24.3%	Cast & Flora, 1998b
		Graphite wrapped with polypropylene mesh		Without heavy metal Current efficiency: 34.1% With heavy metal Current efficiency: 27.5%	

Table 2.6: A summary of results obtained by various workers in bio-cathode denitrification (Continued)

Cell configuration	Electrode material		Experimental Conditions	Results	References
	Anode	Cathode			
Anode is surrounded by biofilm cathode	Carbon	carbon	Flowrate: 2.65 L/d Recycle rate: 1.14 L/min Initial NO_3^-N: 20 mg/L Applied current: 0-100mA pH 7.0	<u>20mA</u> Nitrate removal: 98% N_2 production: 98.95% <u>100mA</u> Nitrate removal: 35% N_2 production: 69.32%	Islam & Suidan, 1998
			Flowrate: 2.65 L/d Recycle rate: 1.14 L/min Initial NO_3^-N: 20 mg/L Applied current: 25mA	Nitrate removal: 82- 87%	
Divided electrolysis cell with porous, water permeable plastic foam	Platinum-coated titanium	Stainless steel and granular activated carbons	Continuous mode HRT: 0.33-6 h Current density: 2.7-20 A/m^2	Current denitrification: 30-90%	Prosnansky et al., 2002

Table 2.6: A summary of results obtained by various workers in bio-cathode denitrification (Continued)

Cell configuration	Electrode material		Experimental Conditions	Results	References
	Anode	Cathode			
Divided electrolysis cell with cationexchange membrane	Dimensionally stable anode (DSA)	Graphite felt	30^0C Applied current: 200 mA pH: 7.0	Nitrate removal: 98%	Park et al., 2005
Divided electrolysis cell with cationexchange membrane and graphite granules	n/a	n/a	pH 7.2 Current density: 23.4 mA/cm^2 Flowrate: 0.35 L/h	Nitrate removal: 74%	Clauwaert. et al., 2009
Divided electrolysis cell with porous sponge foam rubber	Pt- coated metal	Titanium	Current density: 0.82 mA/cm^2 HRT: 6 h pH: 6-7	Current efficiency: ~70%	Sakakibara & Nakayama, 2001

Table 2.6: A summary of results obtained by various workers in bio-cathode denitrification (Continued)

Cell configuration	Electrode material		Experimental Conditions	Results	References
	Anode	Cathode			
Divided electrolysis cell with cationexchange membrane	Titanium plate coated with platinum-iridium oxide (2mm thick)	Graphite plate (5mm thick)	Continuous mode 25-27^{0}C Water velocity: 0.11 m/h Initial NO_3^-N: 21-27 mg/L Applied current: 40-100mA	40 mA Nitrate removal: ~26% Nitrite generated: ~3.5mg/L 50 mA Nitrate removal: ~29% Nitrite generated: ~2mg/L 60 mA Nitrate removal: ~48% Nitrite generated: ~0.2mg/L 70 mA Nitrate removal: ~81% Nitrite generated: ~0.2mg/L 80 mA Nitrate removal: ~78% Nitrite generated: ~3.5mg/L 90 mA Nitrate removal: ~75% Nitrite generated: ~3.0 mg/L	Szekeres et al., 2001

CHAPTER 3: MATERIALS AND METHODS

Before starting the experiments, palm shell granular activated carbon (GAC), carbon and graphite felt have to be washed by synthetic wastewater to eliminate the adsorption effect. Denitrifying bacteria from brewery factory was acclimated and immobilized on the GAC surface. Then, electric current was applied to allow the bacteria adapted with the system. For comparison of anode materials, the electrode spacing, electric current and HRT were set at 3 cm, 10 mA and 6 hours. The screening ranges for the three parameters were fixed at 0.2 to 5.5 cm, 0 to 25 mA and 3 to 60 hours, respectively. Response surface methodology (RSM) would be used after obtaining the suitable parameters range.

3.1 Preparation of electrodes

Palm shell granular activated carbon (GAC) having a porosity of 66.5% was applied as the cathodic material and support for auto hydrogenotrophic biofilm (GAC was found from Bravo Green Sdn. Bhd., Sarawak, Malaysia). The GAC was sieved to a size ranging from 1.4 to 2.0 mm. Prior to its application, it was washed with 0.02 M sulphuric acid (H_2SO_4) and deionized water several times to eliminate dust and contaminants and then dried in an oven at 105^0C for 24 hours in order to eliminate surface moisture. To eliminate adsorption effect of GAC, it was washed by synthetic wastewater.

Titanium (porosity was not measured), stainless steel mesh (hole size 2mm x 2mm), nano-crystalline lead (IV) oxide (PbO_2), carbon felt and graphite felt (both felts had a porosity of 0.95) were applied as anode materials. Each electrode possessed an area of 25cm^2 (5 cm × 5 cm). Titanium was purchased from Sigma-Aldrich where as carbon and graphite felts were provided by SGL Carbon Ltd and stainless steel mesh was delivered by Power Hardware & Trading. Titanium and stainless steel mesh were

polished by means of fine grade (P100) sand paper to eliminate the pollutions and the saturated with 0.02 M H_2SO_4 and rinsed with deionized water. Same pre-dealing phase was faced by carbon and graphite felts but the polishing step was excluded. Moreover, they have faced similar saturation treatment as the GAC had received.

The nano-crystalline PbO_2 were prepared in University of Southampton and the preparation procedure as documented in the literatures (Sirés et al., 2008; Sirés et al., 2010). In brief, a galvanostatic method was used to deposit PbO_2 coatings on various carbon/polyvinyl-ester composite substrates in aqueous methane sulphonic acid electrolytes. A small, cylindrical, undivided cell containing $80cm^3$ of solution was applied effectively to serve the purpose. The cell was magnetically stirred at 300 rpm with a PTFE-coated cylindrical stirring bar (4.5 cm length × 0.8 cm diameter) to ensure reproducible mass transport conditions. The mean thickness of the PbO_2 coatings was 1.9 and 18 μm at 5 and 50 mA cm^{-2}, respectively (these were the only current densities used during the course of the preparation process) (Sirés et al., 2010).

3.2 Reactor configuration

The reactor was constructed from Plexiglas, having 9.5 cm inner diameter and 30cm height as demonstrated in Figure 3.1. Stainless steel rods 5 cm in height were screwed on to an aluminum plate which was installed at the bottom of the reactor to provide better electrical distribution to the GAC. The anode was hung in the reactor by fitting it to four stainless steel support rods. The top and bottom stainless steel rods in the reactor were connected to a programmable direct current (DC) power supply (RS Components, England). The GAC was then immersed in a 2% agar solution at a boiling temperature and left to cool before being installed into the reactor to deliver an adhesive surface on the GAC for accelerating the growth of the autohydrogenotrophic biofilm.

The cathodic bed was created with a height of 8 cm with GAC from the bottom of the reactor.

3.3 Experimental setup and procedure

A mixed culture containing denitrifying bacteria was found from an up-flow anaerobic sludge blanket (UASB) reactor at a brewery factory located in Selangor, Malaysia. The steps to acclimate and immobilize the denitrifying bacteria (that considered of both nitrate and nitrite reductase enzymes) were derived from the procedures of Ghafari et al. (2009a and 2009b). The mixed culture was inserted in a feeding solution consisting 1.250 g $NaHCO_3$/L, 0.650 g K_2HPO_4/L, 0.170 g KH_2PO_4/L, 0.1 g $MgSO_4$/L, 0.027 g $CaCl_2$/L which are the essential nutrients for bacterial growth. 20 mg NO^-_3-N/L (0.122 g $NaNO_3$/L) was added in the feeding solution so that the bacteria could adapt itself with the nitrate that was exist in the synthetic wastewater. The initial pH of the feed solution was around 8.0 and adjusted by carbon dioxide gas to 7.2 and then purged with nitrogen gas to expel the dissolved oxygen. After nitrate reduction reached a steady state, hydrogen gas was sparged once per day in the evenings until the pH of the solution increased from 7.2 to 7.8; while feeding solution was taken out in the mornings. The end of acclimation mixed culture stage was determined by nitrate depletion within 24 hours (Ghafari et al., 2009a).

The immobilized states had been illustrated by Ghafari et al, (2009b) where the acclimated mixed culture was pumped into reactor and permitted to attach on the sticky GAC surface. The feeding solution was pumped into reactor and left for one month to form biofilm. After formation of the biofilm, the denitrifying bacteria completely covered the mesopore and the micropore of the GAC thereby negating any adsorption of nitrate or organic matter in the activated carbon itself (Zhou et al., 2007). Then, the synthetic wastewater (0.25 g $C_6H_{12}O_6$/L, 0.122 g $NaNO_3$/L, 1.250 g $NaHCO_3$/L, 0.650 g

K_2HPO_4/L, 0.170 g KH_2PO_4/L, 0.1 g $MgSO_4$/L, 0.027 g $CaCl_2$/L) was pumped continuously at an HRT of 24 hours for 10 days (after formation of the biofilm) while no electricity was employed in the reactor to allow the mixed culture for adapting with the synthetic wastewater. Before commencement of experiments, synthetic wastewater at neutral pH (without being sparged with hydrogen) was delivered continuously in 3 days at an electric current of 10 mA that was optimum current for denitrifying bacteria survival (Ghafari et al., 2009b; Ghafari et al., 2010).

As shown in Figure 3.1, the synthetic wastewater was kept in a 2 L storage tank and sparged with pure nitrogen gas for 15 minutes to exclude oxygen in the solution since the denitrification process more effectively under anaerobic circumstances (Krul, 1976). Then, the pH of synthetic wastewater was adjusted to 7.2 using carbon dioxide gas before being pumped into the reactor.

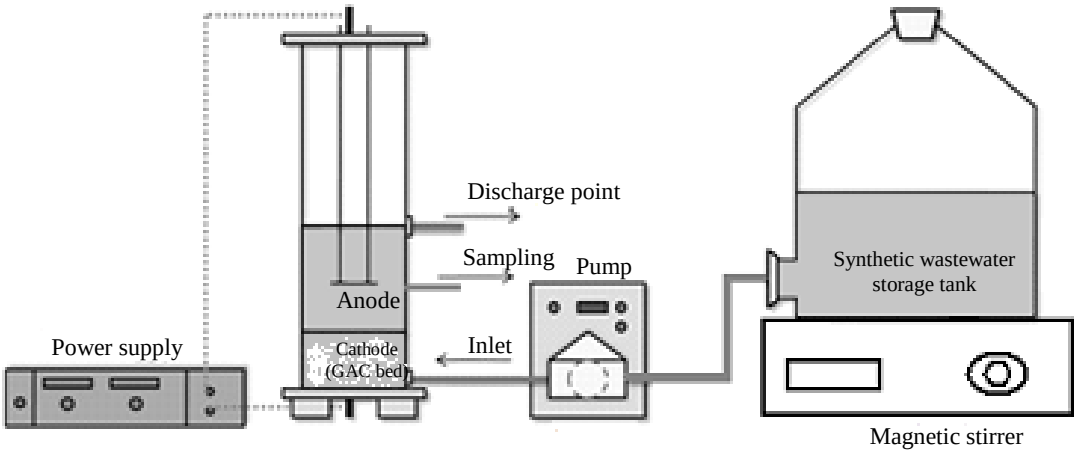


Figure 3.1: Schematic representation of the experimental set-up

3.4 Comparison of anode materials

All the anode materials were hung with four stainless steel support rods and the electrodes spacing, electric current and HRT were set at 3 cm, 10 mA and 6 hours. Before initiate with a new experiment, the solution inside the reactor was purged with pure nitrogen gas for 15 minutes before discharged it out. This is only to ensure that no

oxygen gas was trapped inside the reactor that generated from previous experiment. 5 ml of samples were taken and used to analysis.

3.5 Screening of parameters range

3.5.1 Screening of electrodes spacing range

After picking up the appropriate anode electrodes from section 3.4, the electrodes spacing was varied between 0.2 cm and 5.5 cm to found the perfect range to use in next objective. The electric current and HRT were set at 10 mA and 6 hours.

3.5.2 Screening of electric current range

To observe the electric current range, the electrodes spacing and HRT were installed at constant value that were 3 cm and 6 hours, respectively. For electric current was set at 0 mA, 5 mA, 10 mA, 20 mA and 25 mA.

3.5.3 Screening of HRT range

The HRT range that selected was from 3 hours to 60 hours; while the electrodes spacing and electric current were at 3 cm and 10 mA, respectively. All the organic matter and nitrate-nitrogen elimination in screening phase was estimated by applying the equation 3.1 and 3.2.

3.6 Analytical methods

Nitrate and nitrite concentration were determined by HPLC (Shimadzu 10A) using a UV detector at 210 nm. A Phenomenex Hypersil column (Thermo Electron Corporation, USA) with an internal diameter of 150 mm × 4.6 mm was packed with 5 µm particles for analytical purposes. Organic compound was analyzed by chemical oxygen demand (COD) method (HACH DRB 200) that is the indirect measurement of the amount of organic compound in water (APHA, 1999). The GAC, titanium and PbO_2 anode surfaces were scanned by means of FESEM (AURIGA, ZEISS) under high

vacuum conditions at an accelerating voltage of 1 kV; graphite and carbon felt surfaces were scanned by means of optical microscopy (DMLS, Leica, Germany) and the image was captured with a camera (DFC 290, Leica, Germany).

Performances of bio-electrochemical reactor can be expressed by means of organic matter, nitrate-nitrogen elimination, current efficiency (CE) and specific denitrification (SD) which is defined as follows (Zhou et al., 2009; Islam and Suidan, 1998):

$$\text{Organic matter removal} = \frac{COD_i - COD_f}{COD_i} \times 100\% \quad (3.1)$$

$$\text{Nitrate-nitrogen removal} = \frac{(NO_3^- - N_i) - (NO_3^- - N_f)}{NO_3^- - N_i} \times 100\% \quad (3.2)$$

$$CE = \frac{[5(X_{in} - X_{eff}) - 3Y_{eff}]}{14I} \times \frac{F}{3600} \times \frac{Q}{1000} \times 10 \quad (3.3)$$

$$SD\ (g/KWh) = \frac{Q \times (X_{in} - X_{eff})}{UI} \quad (3.4)$$

where COD_i and COD_f are initial and final COD value (mg/L), $NO_3^- - N_i$ and $NO_3^- - N_f$ demonstrate initial and final nitrate-nitrogen concentration (mg/L), X_{in} and X_{eff} are influent and effluent concentration of nitrate-nitrogen (mg/L), Y_{eff} is the effluent concentration of nitrite-nitrogen (mg/L), *I* is the current supplied (mA), *F* refer to the Faraday's constant (96485 C/mol) and *Q* is the electrolyte flowrate (mL/h), *U* is the voltage (V), 5 and 3 are the stoichiometric coefficient for nitrate and nitrite and 14 is nitrogen equivalent mass (mg eq^{-1}).

3.7 Experimental design

The three most significant process parameters are electrode spacing, electric current and HRT and these are demonstrated as X_1, X_2 and X_3, correspondently. The statistical design and data analysis were carried out by Design-Expert Software (version

8.0.7.1). The central composite design (CCD) method was selected to decrease the number of tests and optimize the effective process variables. The number of experiments designed by CCD at five levels was 17 runs including 3 repeated experimental runs at the central point to eliminate errors and curvature.

The three response functions (nitrate-nitrogen and organic matter removal percentage, nitrite nitrogen concentration) were predicted and expressed follows a quadratic equation as given by Equation (3.5).

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_{i_{i<j}}^{k} \sum_{j}^{k} \beta_{ij} X_i X_j + e \quad (3.5)$$

Where Y is the predicted response, i is the linear coefficient, j is the quadratic coefficient, β is the regression coefficient, k is the number of factors studied and optimized in the experiment and e is the random error. The statistical analysis and adequacy test was carried out by analysis of variance (ANOVA). The statistical significance was tested by the F-test in the same program and the approvable model terms were based on a probability value with 95% confidence level.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Comparison of anode material on organic matter and nitrate removal

Titanium, stainless steel, lead (IV) oxide, graphite and carbon felt are the common electrode materials due to its cheap and availability. Titanium and stainless steel mesh showed the worst performance amongst all the tested anodes; merely 9% and 13% of COD was removed at the end of the process (Figure 4.1). This is because both materials were 'active' electrodes due to which there is a strong interaction between electrode and hydroxyl radicals ($^{\cdot}OH$), causing oxygen transfer from the hydroxyl radicals to the anode surface resulting in the formation of other compounds rather than organic matter oxidation (Alfaro et al., 2006). This result was similar with that reported by Cañizares et al, (2002) who observed the difference of active (stainless steel thin film) and non-active electrode (diamond thin film) in oxidation of aqueous acid phenolic waste. They found that 'non-active' electrode was more advanced since it caused a weak interaction between the electrode and hydroxyl radicals, so the radicals could rapidly react with the waste. Thus the 'active electrode' surface would combine with the radicals to form other higher molecular weight compounds.

The COD elimination of applying titanium was inferior than that for stainless steel mesh since titanium's standard electrode potential (V versus SHE) was lower than that of stainless steel (stainless steel being an alloy of iron, chromium and nickel). Thus, stainless steel mesh could oxidize better than titanium. Moreover, Feng et al, (2003) had proved that no hydroxyl radicals were detected on titanium surfaces by analyzing with *p*-nitrosodimethylaniline (RNO). This could be because titanium had a propensity to generate oxygen from the electrolysis of water, thereby negating the chances of hydroxyl radical formation. Moreover, the physical characteristics of titanium was not similar with others applied in this work since titanium was a solid electrode while other

anodes were porous or mesh like in nature (Figure 4.2 a-d). The porous and mesh structure could provide a large active surface area and could boost the formation of hydroxyl radicals (·OH).

Nano-crystalline PbO_2, carbon and graphite felt are 'non-active' electrodes, but PbO_2 had the highest oxidation power and was able to generate reactive hydroxyl radicals {PbO_2(·OH) } (Eq. 4.1 and 4.2) that result the overall organic matter oxidation (Sirés et al., 2008; Sirés et al., 2010).

$$PbO_2 + H_2O \rightarrow PbO_2(\cdot OH) + H^+ + e^- \quad (4.1)$$

$$\text{Organic matter} + PbO_2(\cdot OH) \rightarrow CO_2 + PbO_2 + zH^+ + ze^- \quad (4.2)$$

The nitrate-nitrogen removal is highly dependent on the pH of the process. As described in Eq. 2.8, the pH of the system tends to increase as hydroxyl ions are generated and higher pH has a common propensity to retard the denitrification process. The carbon dioxide produced from the anodic oxidation of organic compounds would transfer to the cathodic part and adjust the pH by dissolving the carbon dioxide into water to form CO_3^{2-} as the final product (as depicted in Eq. 4.3-4.5) (Freguia et al, 2008). However, the electrolytic ohmic loss in transferring carbon dioxide between anodic and cathodic sides has to be minimized by decreasing the migration distance (Rozendal et al., 2008).

$$CO_2 + H_2O \rightarrow H_2CO_3 \quad (4.3)$$

$$H_2CO_3 + OH^- \rightarrow H_2O + HCO_3^- \quad (4.4)$$

$$HCO_3^- + OH^- \rightarrow H_2O + CO_3^{2-} \quad (4.5)$$

In comparison to the different anodes studied in this work, PbO_2 demonstrated the highest removal of organic matter and nitrate-nitrogen. This was in accordance with the literature (Sirés et al., 2010; Flox et al., 2009; Zhou et al., 2011). The other part of

this research involved investigating parameters range and the usage of response surface methodology to determine optimum reactor conditions for simultaneous removal of both organic matter and nitrate.

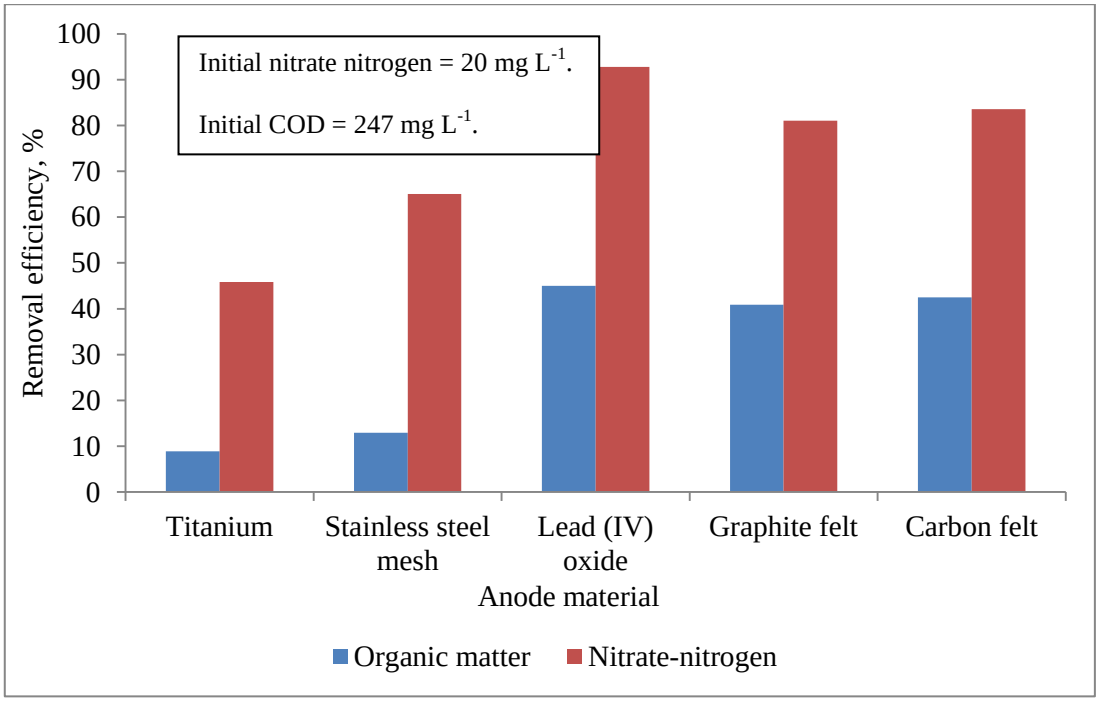


Figure 4.1: Organic compounds and nitrate-nitrogen removal with different types of anode material.

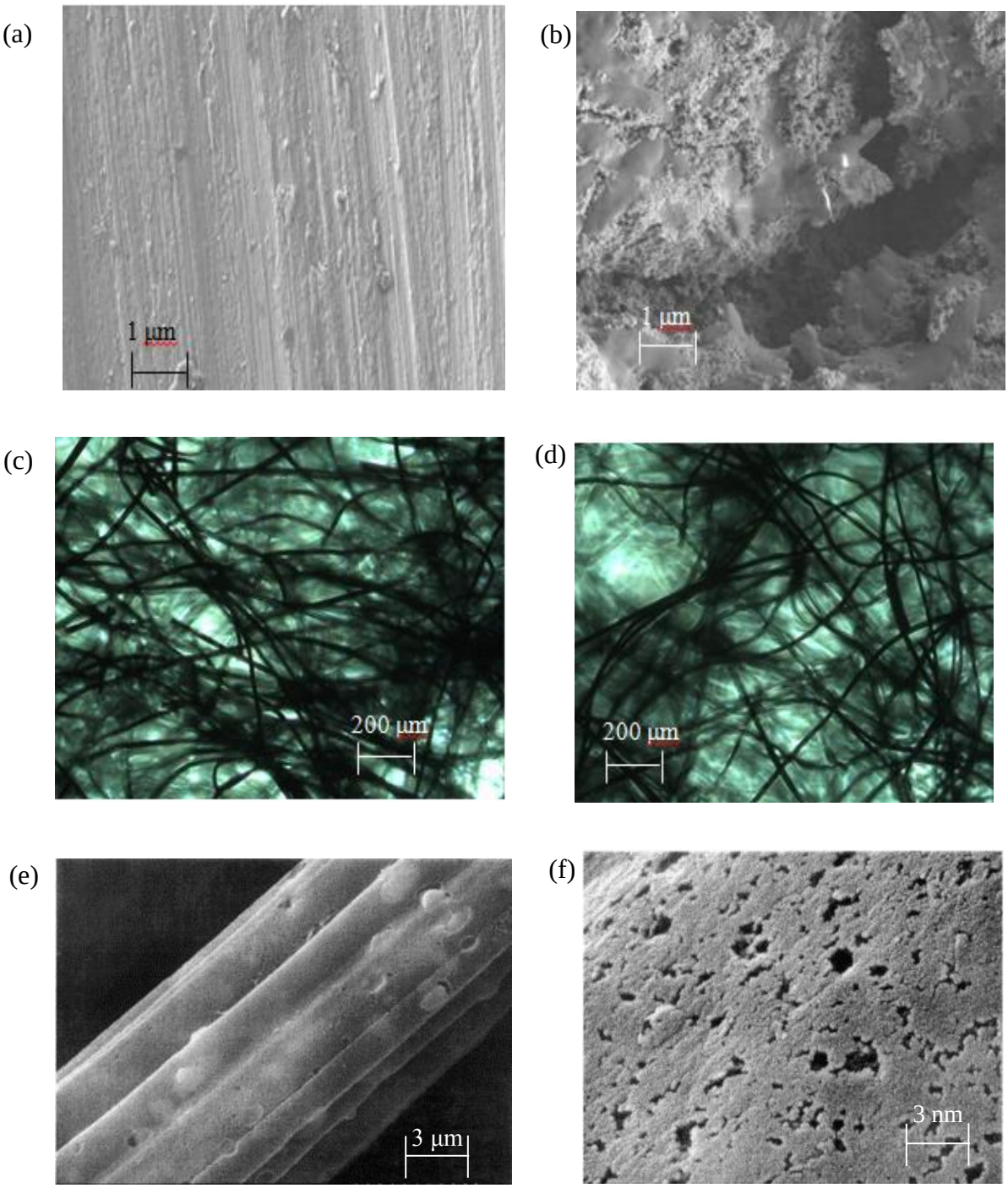


Figure 4.2 (a) FESEM image of titanium (10,000 x). (b) FESEM image of lead (IV) oxide (10,000 x). (c) Microscope image of graphite felt (100 x). (d) Microscope image of carbon felt (100 x). (e) FESEM image of GAC (10,000 x). (f) FESEM image of GAC (100,000 x)

4.2 Screening of different parameters range

4.2.1 Screening of electrodes spacing range

Figure 4.3 demonstrated that the organic matter elimination boosted by rising up the electrodes spacing from 0.2cm to 3cm. When the electrode distance was getting small, oxygen could cross over and form by-products such as hydrogen peroxide that could decrease the effectiveness of the treatment process (Behera et al., 2010; Cheng et al., 2006; Pham et al., 2009). H_2O_2 can be produced through oxygen reduction reaction (ORR) (Sánchez-Sánchez and Bard, 2009). These compounds accumulated around the electrode surface and would decrease the power per surface area of anode; hence it could reduce the effectiveness of the treatment process (Cheng et al., 2006). Most of the researchers inserted a membrane between the electrodes to avoid the oxygen cross over crisis. However this would increase the electrolyte resistance and create fouling problem resulted failure to improve the removal efficiency.

Nevertheless, the removal efficiency of organic matter decreased during the time when electrodes spacing boosted from 3 cm to 5.5 cm. This could have been due to an increasing internal resistance when electrode spacing was increased thereby requiring an extra driving force (electric potential) to accomplish the process. The result was same to the work of Cheng & Logan, (2011) who confirmed that internal resistance could be reduced by reducing the electrodes spacing between anode and cathode.

There was approximate 100% of nitrate-nitrogen elimination when the electrodes spacing was between 0.2 cm and 0.5 cm. This happened because of carbon dioxide from organic matter oxidation are easily migrated to cathodic side to adjust the pH since the internal resistance had been reduced by the decreasing with electrodes spacing. In the review of Rozendal et al (2008), internal resistance was reduced with the electrodes spacing since the movement of carbonate ions, bicarbonate ions and carbonic acid through electrolyte to the cathodic side was easily. As the electrodes spacing rose

from 0.5 cm to 5.5 cm, the nitrate-nitrogen removal efficiency was reduced from 99% to 84%. This could be explained by internal resistance factor as well where it increased when the distance between anode and cathode became far apart. Despite carbon dioxide produced higher in 3cm electrodes spacing, carbonate and bicarbonate ions would react with H^+ in the electrolyte (Eq. 4.6 and 4.7) rather than transfer to the cathodic side to react with hydroxyl ions since internal resistance is higher.

$$CO_3^{2-} + H^+ \leftrightarrow HCO_3^- \quad (4.6)$$

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \quad (4.7)$$

From the outcome found, the appropriate range of electrodes spacing for response surface methodology (RSM) was between 0.5 cm and 5.5 cm. The organic matter and nitrate-nitrogen elimination efficiency only deviated around 2% between 0.2 cm and 0.5 cm, so 0.2 cm electrodes spacing could be removed. By comparing 0.5 cm to 3 cm and 3 cm to 5.5 cm, nitrate-nitrogen elimination was poorer at 3 cm to 5.5 cm and might be become unchangeable after 5.5 cm. Thus, the maximum electrode spacing was 5.5 cm as during the time of the range when it is too wide would impact the action of RSM.

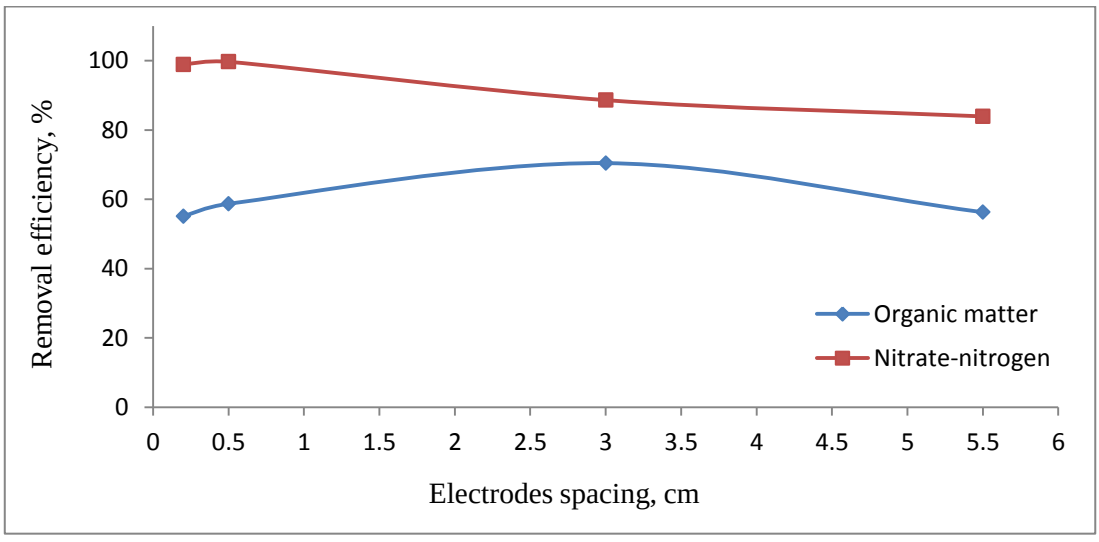


Figure 4.3: Organic matter and nitrate-nitrogen removal efficiency at different electrodes spacing.

4.2.2 Screening of electric current range

The plot in Figure 4.4 depicted that with the rise of applied current more organic matter were eliminated. The rise in current promoted the generation of hydroxyl radicals that mineralized the organic matter as limned in Eq. 4.1 and 4.2. Notwithstanding, the elimination of organic compounds was observed to decrease at an electric current beyond 10mA. This could be as a result of very high electric currents could enhance the anodic water oxidation process to generate oxygen instead of hydroxyl radical; hence oxidation of organic matters was negatively impacted. This outcome was akin to Zhou et al. (2009) who using bio-electrochemical reactor to deal with groundwater pollutant. They found that the highest total of carbon removal was at 23 mA and afterward no reduction because oxygen formed on the anode after the optimum electric current value.

The nitrate-nitrogen elimination tends to be same to organic matter removal plot. When electric current was increased, the nitrate-nitrogen elimination also improves potentially, but at higher electric currents the elimination figures are retarded. This could be because when applied current is swelled, water electrolysis occurs resulting in the generation of sufficient hydrogen to increase the removal rate of nitrate. In additional, more carbon dioxide could be generated at higher electric current on anodic side to adjust the pH of the system. However, after 10 mA, nitrate-nitrogen removal was reduced significantly since excessive hydrogen concentration caused channeling in GAC beds and forms dry spaces which inhibit the denitrification performance (Szekeres et al., 2001). Moreover, high electric current also generates higher hydroxyl ions through the hydrolysis of water at the cathode thereby shifting the pH of the system towards the alkaline region. This has a negative impact on the nitrate-nitrogen removal rate (Zhou et al., 2007).

Since organic matter and nitrate-nitrogen elimination not attained constant at any electric current value, the selection of maximum current value should depend on the removal efficiency that decreases continuously for obtaining a dome shape in RSM. Dome shape limns the removal efficiency will rise to a maximum value then reduces afterward. By comparing 10 mA to 20 mA and 20mA to 25 mA, the eliminations trend for both contaminants were reduced continuously but later part could be removed as wider range would affect the precision to obtain the optimum value through RSM.

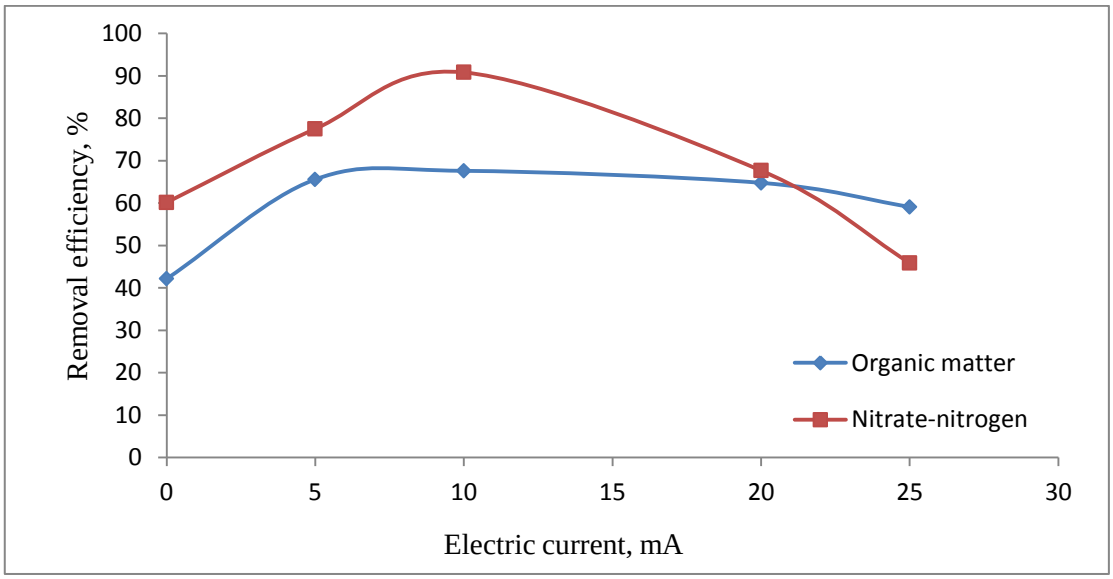


Figure 4.4: Organic matter and nitrate-nitrogen removal efficiency at different electric current

4.2.3 Screening of HRT range

The lowest organic matter and nitrate-nitrogen elimination was at HRT 3 hours because HRT was inversely proportional to the flow rate as showed in equation 4.8. The flow rate was reduced thereby decreasing the organic compound loading. So, there was sufficient reaction time for the hydroxyl radicals with organic matter.

$$\text{HRT (h)} = \frac{\text{Volume of reactor (cm}^3\text{)}}{\text{Flowrate } \left(\frac{\text{ml}}{\text{h}}\right)} \qquad (4.8)$$

At low HRT, both contaminants did not have sufficient treatment time to treat vast quantity pollutants before discharged to the effluent. So, the removal efficiency for organic matter and nitrate-nitrogen was merely 43% and 24%, respectively. As HRT rose, the both removal efficiency were increased respectively since the flowrate was decreased and they had enough reaction time before being out to the ecosystem. After 48 hours, both contaminants removal efficiency was became constant. This could be described by they had successfully attained equilibrate with required reaction time. The result tend to be same to the research of Ghafari et al. (2009a) and Zhou et al. (2009a) who proved that low HRT was failure to treat nitrate-nitrogen and total organic carbon because of the higher loading rate. The selection of optimum HRT is also based on some core parameters such as wastewater compositions, reactor configuration, quantity of bacteria involved, electric current applied and electrodes spacing.

The removal efficiency of organic matter and nitrate-nitrogen in 3 to 6 hours is diverse around 18% and 70%, respectively. Thus, it was presumed that the removal efficiency for both contaminants reduced incessantly from 0 to 3 hours. Consequently, 0 to 3 hours was taken as the range of RSM for attaining a dome shape. The organic matter and nitrate-nitrogen removal efficiency between HRT 48 and 60 hours only deviated 0.4 %, so the HRT after 48 hours could be eliminated. Thus, the appropriate range for HRT used in RSM was 0 to 48 hours.

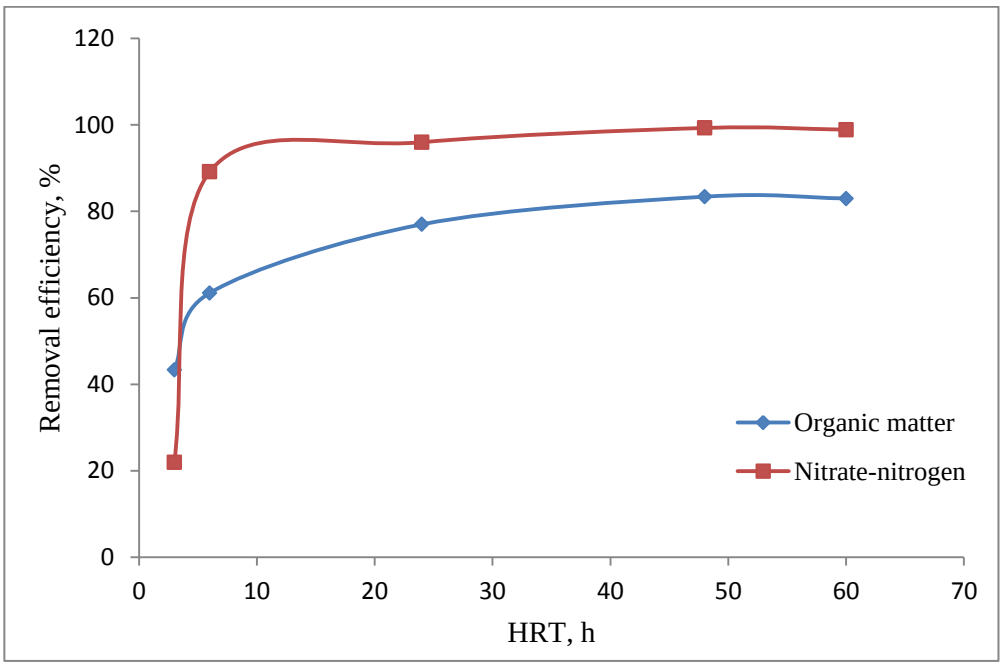


Figure 4.5: Organic matter and nitrate-nitrogen removal efficiency at different HRT

4.3 Modeling and optimization using response surface methodology (RSM)

4.3.1 Development of regression equations and statistical analysis

The objective of this part was to generate a model by using RSM and observe the interaction between parameters by applying the screening range which found in section 4.2. The correlations between the process variables and elimination of organic matter, nitrate-nitrogen and nitrite-nitrogen accumulation were tested using central composite design (CCD). The coded values for these variables were set at 5 levels (-1 (minimum), -0.5, 0 (central), +0.5 and 1 (maximum)). The minimum code (-1) for HRT showing a value of zero hours was impossible, so it was replaced by the code of -0.75 (6 h) since it is the intermediate code between -1 and -0.5. The analysis suggested a quadratic model for organic matter and nitrate-nitrogen elimination and a 2FI model for nitrite-nitrogen concentration. Table 4.1 demonstrated the design of experiment as well as the experimental results. The final regression model equation in terms of coded and actual factors after removing the non-significant terms for three responses are depicted in Table 4.2.

Table 4.1: Central composite design for the experiments and response results

Run	Electrodes spacing (cm), X_1	Electric current (mA), X_2	HRT (h), X_3	Organic compound removal (%)	NO_3^--N removal (%)	NO_2^--N concentration (mg/L)	Current efficiency, CE (%)	pH	Specific denitrification (g/ KWh)
1	0.50	0.00	48.00	83.81	99.75	0.08	-	9.64	-
2	3.00	10.00	36.00	76.11	99.61	0.18	52.26	9.33	18.30
3	0.50	0.00	6.00	29.96	51.11	0.10	-	9.16	-
4	5.50	20.00	48.00	63.56	98.06	0.28	19.23	9.87	5.48
5	5.50	0.00	48.00	81.38	99.54	0.57	-	9.20	-
6	3.00	10.00	12.00	76.11	99.55	0.08	157.14	9.66	54.86
7	3.00	15.00	24.00	73.68	99.67	0.13	52.38	9.81	15.70
8	3.00	5.00	24.00	70.85	99.72	0.13	157.21	9.60	63.66
9	0.50	20.00	48.00	75.71	96.72	0.01	19.13	8.90	5.41
10	0.50	20.00	6.00	67.61	88.26	0.01	139.63	8.78	39.44
11	1.75	10.00	24.00	71.66	99.27	0.18	78.13	9.93	27.35
12	5.50	0.00	6.00	37.25	57.97	0.30	-	10.17	-
13	3.00	10.00	24.00	73.68	94.35	0.15	74.29	9.23	26.00
14	4.25	10.00	24.00	69.23	99.64	0.25	78.25	10.19	27.46
15	3.00	10.00	24.00	75.30	100.00	0.14	78.80	9.10	27.56
16	3.00	10.00	24.00	76.11	99.61	0.18	78.39	10.03	27.45
17	5.50	20.00	6.00	58.30	83.49	0.02	132.03	9.07	37.31

Table 4.2: Regression models

Source	Models in terms of coded (Y_{cod}) and actual (Y_{act})
OM	$Y_{cod} = 74.14 – 2.10X_1 + 5.44X_2 + 10.76X_3 - 3.29X_1X_2 - 12.06X_2X_3 - 16.93X_1^2 - 9.64X_2^2 + 17.04X_3^2$
	$Y_{act} = 27.46696 + 16.72839X_1 + 4.07335X_2 – 0.46937X_3 – 0.13158X_1X_2 – 0.050255X_2X_3 – 2.70848X_1^2 – 0.096406X_2^2 + 0.029589X_3^2$
NO_3^--N	$Y_{cod} = 99.76 + 0.40X_1 + 7.96X_2 + 14.92X_3 - 9.52X_2X_3 - 17.00X_2^2$
	$Y_{act} = 49.88051 + 0.16054X_1 + 5.14745X_2 + 1.01828X_3 - 0.039650X_2X_3 - 0.17000X_2^2$
NO_2^--N	$Y_{cod} = 0.16 + 0.11X_1 - 0.085X_2 + 0.075X_3 - 0.052X_1X_2 + 0.078X_1X_3$
	$Y_{act} = 0.071503 + 0.033234X_1 - (2.34415E\text{-}3)X_2 - (7.75279E\text{-}4)X_3 - (2.06712E\text{-}3)X_1X_2 + (1.29571E\text{-}3)X_1X_3$

X_1 is electrodes spacing, X_2 is electric current and X_3 represent HRT

ANOVA is utilized to check the significance and adequacy of the model. The results of the ANOVA for the three systems are described in Table 4.3. The F-value of the model for three systems was significant and these had a 0.01% chance that a "Model F-Value" this large could occur due to noise. Generally, values of Prob. > F being less than 0.05 would be classified as significant effect. It was obtained that for *F* values greater than 0.10 the model terms were not significant. The "F-value of Lack of Fit" for models was 11.55, 2.61 and 3.98 implying that the lack of fits was not significant relative to the pure error. Non-significant lack of fit was desirable and depicted that the model was suitable to the predicted response variables within the range of parameters studied.

The quality of the model was evaluated based on the correlation coefficient value. The predicted R^2 and adjusted R^2, tow quality measurement of the models prediction of the response value and the amount of variation about the mean as described by the model, respectively were assessed. From the literature, the predicted and adjusted R^2 should be within approximately 0.20 of each other to be in reasonable

agreement. If they are out range, there may be a trouble with either the data or the model (Nosrati et al., 2011). In all the cases under observation, the predicted R^2 and adjusted R^2 were in reasonable agreement. Based on the presented ANOVA test results, the model usage described the simultaneous bio-electrochemical denitrification and organic matter removal quite well and can be applied potentially to navigate the design space.

Table 4.3: ANOVA and R-squared (R^2) statistics for fitted model

	F	P	LOF F	LOF P	R^2	Adj. R^2	Pred. R^2	AP
OM	29.56	<0.0001	11.55	0.0818	0.9673	0.9345	0.7674	18.752
NO_3^--N	28.67	<0.0001	2.61	0.3075	0.9287	0.8963	0.8408	17.917
NO_2^--N	39.38	<0.0001	3.98	0.2169	0.9471	0.9230	0.9261	24.456

OM: Organic matter; F: F-value; P: Probability of error; LOF: Lack of fit; AP: Adequate precision.

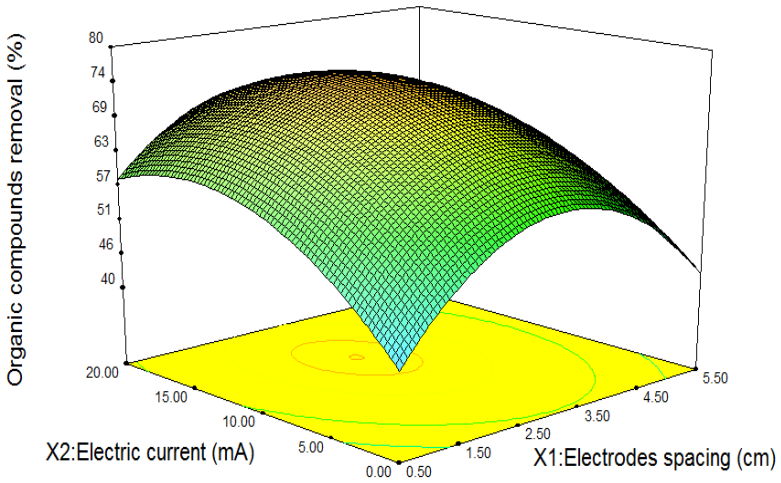
4.3.2 Effect of parameters on organic matter and nitrate-nitrogen removal

RSM was applied to observe the connection of three variables on organic matter and nitrate-nitrogen elimination and accumulation of nitrite-nitrogen. Three-dimensional plots were produced depend on the regression model in Table 4.2. Figure 4.6, 4.7 and 4.8 explained the response surface plot for organic matter and nitrate-nitrogen removal and nitrite-nitrogen concentration upon completion of experiments.

The response surface plot in Figure 4.6(i) meant that the joint impact of electrodes spacing and electric current on organic matter removal efficiency at constant HRT 24 hours. The increasing of electrodes spacing and electric current would improve the organic matter elimination. However, when electrodes spacing and electric current were too high, it would reduce the removal efficiency since higher internal resistance was produced and competitive electric current utilized to form oxygen and hydroxyl radical. The effect of electric current and HRT on organic matter concentration at

constant electrodes spacing (3 cm) was indicated in Figure 4.6(ii). Low HRT and electric current could not eliminate organic matter vastly for the inadequate reaction time and lack of enough quantities of hydroxyl radicals. However, at higher HRT and electric current, oxygen was formed prior to hydroxyl radicals even though there was enough reaction time. Moreover, longer HRT meant higher cost due to lower specific denitrification (Table 4.1) as electric current had to be maintained for a longer time. Hence, HRT was found to be most important parameter compared with other variables.

(i)



(ii)

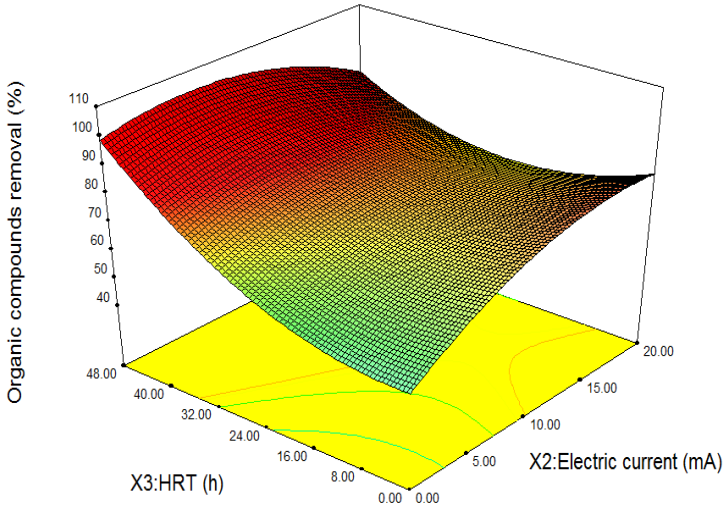


Figure 4.6: Response surface plot for organic matter removal (i) interaction between electrodes spacing and electric current. (ii) interaction between electric current and HRT.

In nitrate-nitrogen elimination process the probability rates of X_1X_2 and X_1X_3 were more than 0.1 which depicted the insignificant and should be eliminated from the model. Figure 4.7 depicted the interaction between applied current and HRT on nitrate-nitrogen elimination at constant electrodes spacing (3 cm). Low electric current and HRT were failed to eliminate nitrate-nitrogen for lower production of hydrogen ions and insufficient reaction time. The rise in electric current and HRT improved the nitrate-nitrogen removal. After arriving at optimum electric current, elimination started to decrease since excessive hydrogen generated that would cause channeling problem in GAC bed despite of sufficient reaction time. Nitrate-nitrogen elimination propensity became flatten (achieved 100 %) after 40 hours at electric current range 10 to 15 mA because there had found sufficient reaction time at 40 hours.

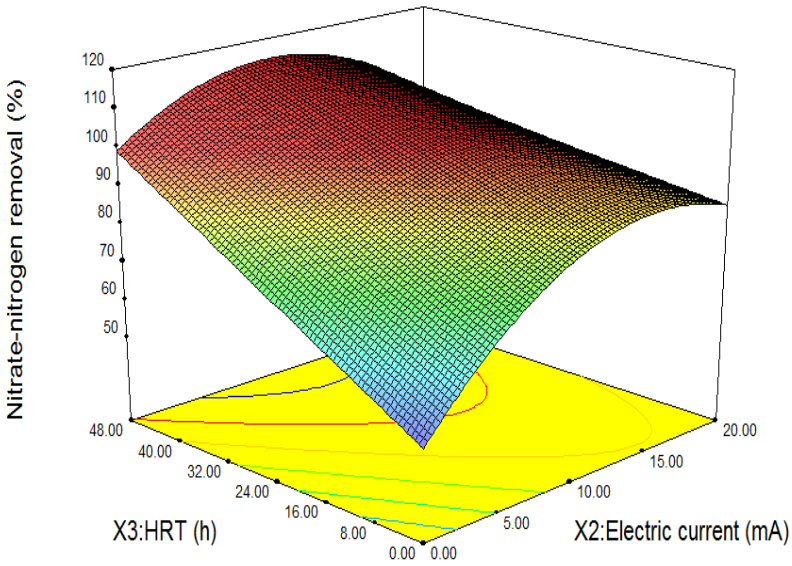


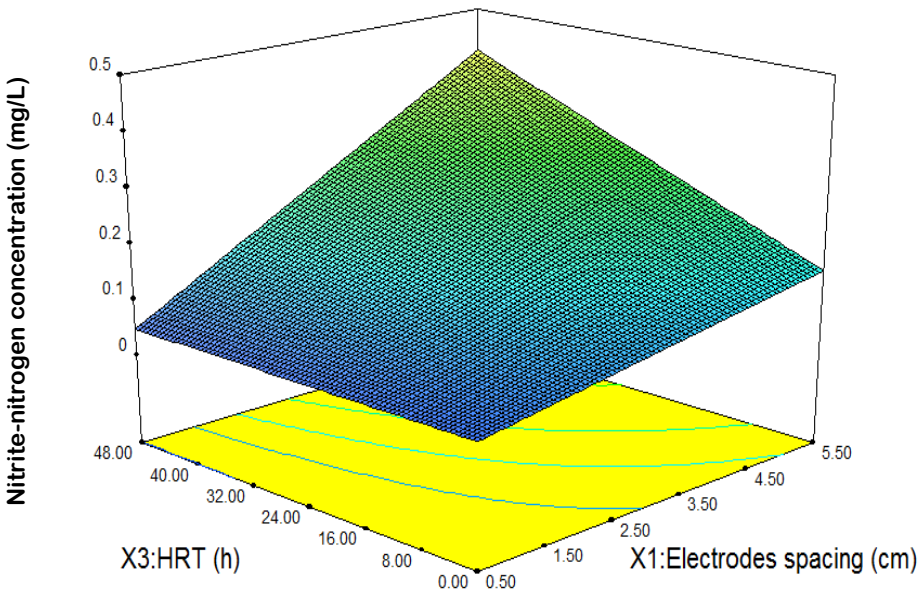
Figure 4.7: Response surface plot for nitrate-nitrogen removal: interaction between electric current and HRT

Nitrite is an intermediate by-product of hydrogenotrophic denitrification (Eq. 2.6 and 2.7) and nitrite reductase is more sensitive than nitrate reductase which indicates that more dissolved hydrogen is required for nitrite reductase to be effective (Chih et al., 1999; Islam et al., 1998). Thus, insufficient pH adjustment will cause accumulation of nitrite especially at pH values of more than 9.0. Figure 4.8(i) depicted

that there was no optimum nitrite accumulation as it kept rising with an increase in electrodes spacing and HRT. The adjusting pH process would be decreased when the electrode spacing is higher since the migration distance of H_2CO_3 towards cathode increase (Eq. 4.3 and 4.4). Hence, H_2CO_3 might take long time towards cathode zone to react with OH^- (Rozendal et al., 2008). Moreover, electrolyte ohmic losses could be decreased by small electrodes spacing distance (Rozendal et al., 2008).

High HRT did not result in the flushing out of excess hydroxyl anions from the cathodic zone thereby resulting in a rise in pH. The relationship between electrode spacing and electric current on nitrite concentration was demonstrated in Fig. 4.8 (ii). Nitrite accumulation was inversely proportional to electric current and directly proportional to the electrodes spacing. This meant that higher amounts of hydrogen ions could react with hydroxyl anions at higher currents and result in a steady pH all throughout the system. Thus, the pH was found to be a significant parameter that could indirectly influence nitrite accumulation in the process.

(i)



(ii)

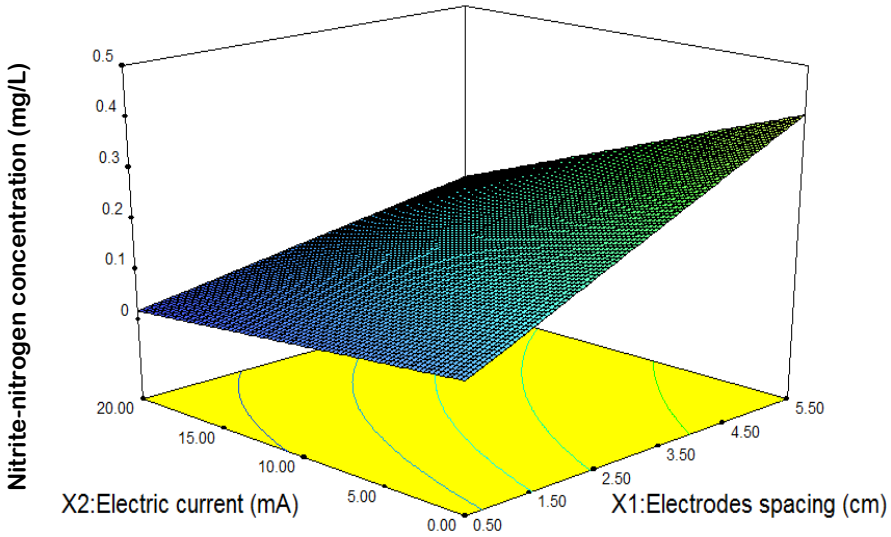


Figure 4.8: Response surface plot for nitrite-nitrogen concentration (i) interaction between electrodes spacing and HRT. (ii) interaction between electrode spacing and electric current.

4.4 Effect of parameters on current efficiency and specific denitrification

Figure 4.9 depicts the current efficiency (CE) and specific denitrification (SD) at different set of parameters (both model predictions as well as experimental values are shown- as explained in the latest section, the model predicted experimental values to within 2% accuracy). It shows that when electric current and HRT are 10 mA and 24 h respectively, CE is unaffected by electrode spacing as proven from Run 11, 13, 14, 15, 16 (Table 4.1) since the values are approximately same. The same trend is found in Run 4 and Run 9 (20 mA, 48 h). Electrode spacing parameter is insignificant for nitrate removal and has been removed in the regression model (Table 4.2). When electrodes spacing and HRT are 3 cm and 24 h respectively, electric current increased from 5 to 15 mA thereby causing a decrease in CE as proven from Run 7, 8, 13, 15 and 16. Longer HRT also leads to lower CE. The CE value of more than 100% is due to the presence of hydrogen gas from previous runs that have been trapped in the GAC bed (Ghafari et al., 2009b). As a whole, higher CE can be achieved by low electric current and HRT,

respectively. These propensities were same to SD as well where electrode spacing was not significantly impacted and low electric current and HRT gave higher SD values.

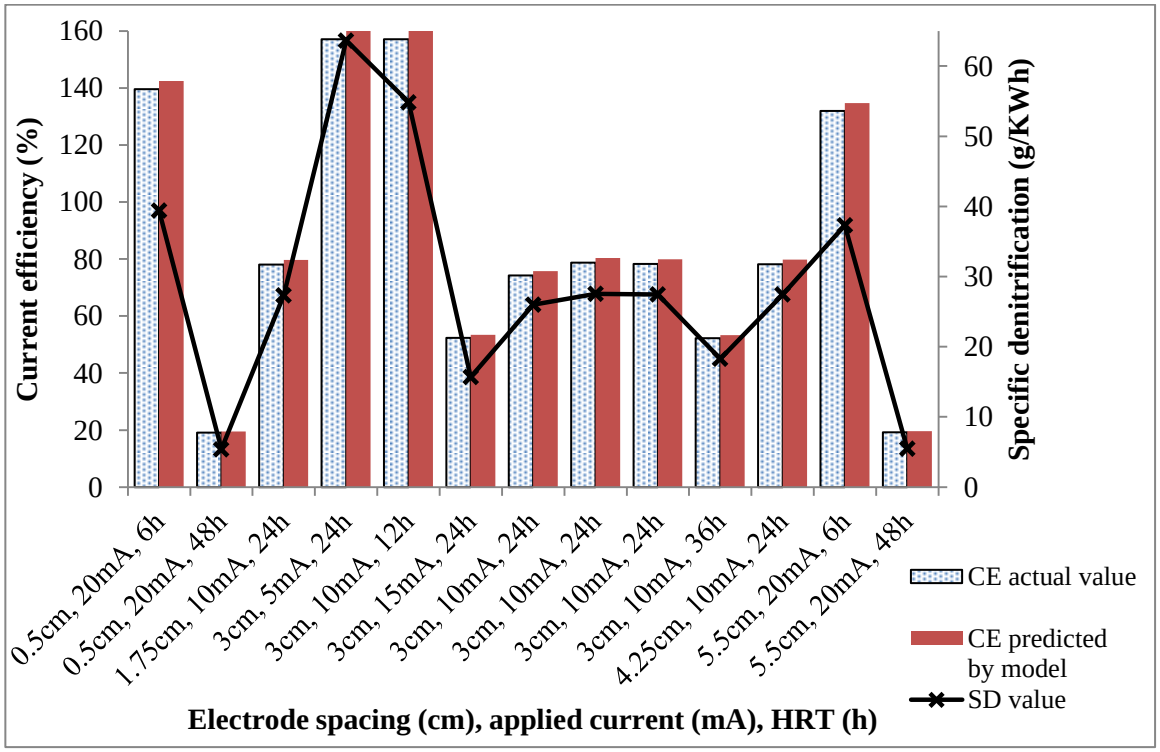


Figure 4.9: CE and SD at different sets of electrodes spacing, electric current and HRT.

4.5 Validation of the model

Data provided in Table 4.3 shows that the models were significant at the 5% confidence level since P-values were fairly less than 0.05 (Ghafari et al., 2009b). The "F-value of Lack of Fit (F. LOF)" for models were 11.55, 2.61 and 3.98, implying that LOFs are not significant relative to the pure error. Whilst seeking for a model to fit the data, non-significant LOF is desirable (Chih et al., 1999). A high R-squared value, close to 1, is desirable and appreciable results (>0.9) are seen for all three models. "Adjusted R-squared" is R-squared adjusted for the number of terms in the model relative to the number of points in the design. It is an estimate of the fraction of overall variation in the data accounted for by the model. "Predicted R-squared" measures the amount of variation in new data described by the model. A reasonable agreement of Adj. R^2 with Pred. R^2 is compulsory and difference should not be greater than 0.2 (20%). Therefore,

obtained results expose again that the data fits the models well. “Adequate Precision” compares the range of predicted values at the design points to the average prediction error (measure of signal to noise ratio). As per the requirement of the models, AP should be greater than 4 in order to depict that the noise is not contributing any error in the response surface (Ghafari et al., 2009c; Beg et al., 2003) and values of 18.752, 17.917 and 24.456 showed that the models din not have any significant error because of noise. Hence, statistical analysis disclosed the sufficiency of the model and developed models can be applied to navigate the design space defined by the CCD.

Finally, to verify the proposed model, experiments were accomplished according to the optimum conditions suggested by RSM which were electrode spacing of 3.2 cm, electric current of 18 mA and HRT of 45 h. The organic compounds and nitrate-nitrogen removal under the optimum condition was 83% and 99%, respectively and the nitrite-nitrogen effluent concentration was 0.026 mg L^{-1}. The removal values proposed by the model were 85% of organic compounds and 100% of nitrate-nitrogen and the percentage error between experimental and predicted results was only 1.0-2.0%. Hence, it can be concluded that the predicted model is sufficient to predict the removal of organic compounds and nitrate-nitrogen under similar circumstances. It also has to be noted that the achieved pollutant removal values are comparable to the ones obtained by other methods using expensive BDD or titanium electrodes as shown in Table 4.4 Virkutyte and Jegatheesan employed a 3L electrodialytic reactor to treat real aquaculture wastewater. The reactor was equipped with platinized titanium rod anode and cathode that were separated by means of a cation and anion permeable membrane. The authors proved that Fenton’s reagent was more advanced for organic oxidation and 97.3% of TOC and 94.8% of nitrate was removed in 48 h by applied current of 30 mA. However, Fenton's reagent is discouraged from being used in wastewater treatment due to the formation of toxic intermediates. Diaz et al, (2011) have used chloride as an

oxidizing agent with boron doped diamond (BDD) electrodes to treat and reuse seawater in recirculating aquaculture systems. They have found that about 88% of COD and approximately 100% of total ammonia nitrogen (TAN) is removed at a current density of 5 mA/cm^2. BDD is considered to be a good material in electrochemical processes due to its feasibility of producing hydroxyl radicals. However, it is very expensive.

Table 4.4: Comparison between different approaches for the removal of organic matter and nitrate

Cell operation and configuration	Electrode material		Type of wastewater	Experimental Conditions	Results	References
	Anode	Cathode				
Divided two electrodes with cation and anion permeable membrane	Ti	Ti	Aquaculture wastewater	Input current: 30 mA pH: 2.2-2.4 Electrolysis time: 48 h Fenton's reagent: 40 mM	TOC removal: 97.3% Nitrate removal: 94.8%	Virkutyte and Jegatheesan, 2009
Divided recirculation batch electrolysis	BDD	BDD	Seawater containing chloride ion used in a Recirculating Aquaculture System	Flowrate: 6 L min^{-1} Current density: 5 mA cm^{-2}	TAN removal: ~100% COD removal: ~88%	Diaz et al., 2011
UBER	PbO_2	GAC	Synthetic wastewater	HRT: 45 h Electric current of 18 mA	Nitrate removal: ~99% COD removal: ~83%	This work

CHAPTER 5: CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

Simultaneous denitrification and organic matter elimination with denitrifying bacteria immobilized on GAC cathode have been conducted in a bio-electrochemical reactor. To date, there are no studies comparing different materials for the simultaneous removal of both pollutants. Of all the anodic materials observed, a nano-crystalline PbO_2 has been chosen as it has the highest organic matter elimination capability at the anode and influences highly efficient denitrification system at the cathode.

Parameters of interest were the electrodes spacing, electric current and HRT. These independent variables were varied and removal efficiencies of the process were determined. Removal efficiency of organic matter was increased but nitrate was vice-versa when the electrode distance was getting bigger. A parabola trend was achieved for both pollutants elimination when the electric current was increased. Removal efficiency for both contaminants was directly proportional to the HRT and became constant after 48 hours. Hence, the electrodes spacing, electric current and HRT range applied to response surface methodology (RSM) after screening were 0.5 cm to 5.5 cm, 0 mA to 20 mA and 0 to 48 hours, respectively.

Under optimum conditions that were suggested by RSM i.e. electrode spacing of 3.2 cm, electric current of 18 mA and HRT of 45 h, about 99% of nitrate-nitrogen and 83% of organic matter were removed. Nitrite is an intermediate by-product of denitrification and it directly proportional to the electrode spacing and HRT but inversely proportional to electric current. Therefore, the supervision and control of the pH values is essential in future studies for preventing nitrite accumulation at high pH values.

5.2 Recommendation

1. In this work, mixed culture was applied to eliminate nitrate-nitrogen at cathodic part. Pure culture can be cultivated from mixed culture which is merit for further study attempts.
2. pH of the process should be controlled in neutral conditions and this might be solved with recycle process that investigated by few researchers.
3. The finding of optimum condition based on this work should be applied in real wastewater.

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APPENDIX A

PROCEDURE FOR CALCULATION POSOSITY OF GRANULAR ACTIVATED CARBON

After sieving GAC into size range of 1.4 -2 mm, they were washed by ultra-pure water and dried in an oven for 24 hours to remove water and surface moisture. 100g of GAC was immersed in ultra-pure water and the overall volume became 200 ml. Then, the solution was stirred for a while to be saturated thoroughly. All the weighting process was carried in 20^0C and assumed that there was no air bubble in thoroughly admixed GAC.

$W_1 = W_{beaker} + W_{GAC} + W_{water} = 330.85g$

After being dried at 105^0C,

$W_2 = W_{beaker} + W_{GAC} = 200.688g$

$W_{water} = W_1 - W_2 = 130.162g$

The water density at 20^0C was 0.98 g/ml,

$V_{water} = W_{water}$ / Water density

= 130.162 / 0.98

= 132.82 ml

$V_{GAC} = 200 - V_{water} = 200 - 132.82 = 67.18$ ml

Porosity, $n = V_v / V_t$, where $V_v = V_{water} + V_{air}$

= 132.82 + 0 (V_{air}: No air in admixed with GAC and water)

= 132.82 ml

$V_t = V_{water} + V_{GAC} + V_{air}$

= 132.82 + 67.18 + 0

= 200 ml

Therefore, $n = V_v / V_t$

= 132.82 / 200

= 0.6641

~ 66.5% (GAC porosity)

APPENDIX B

CALIBRATION CURVE OF HPLC

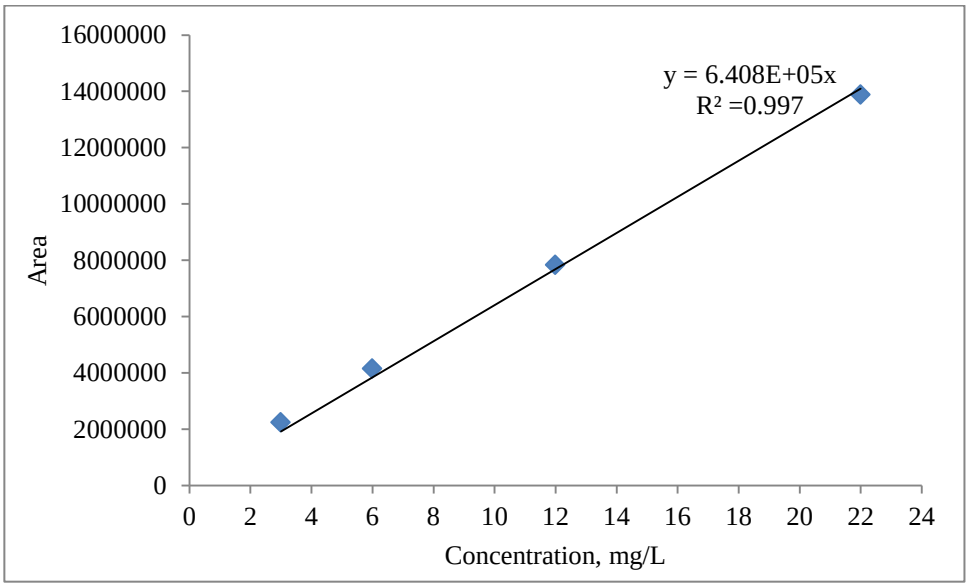

Figure B1: Calibration curve for nitrate-nitrogen standards obtained by HPLC

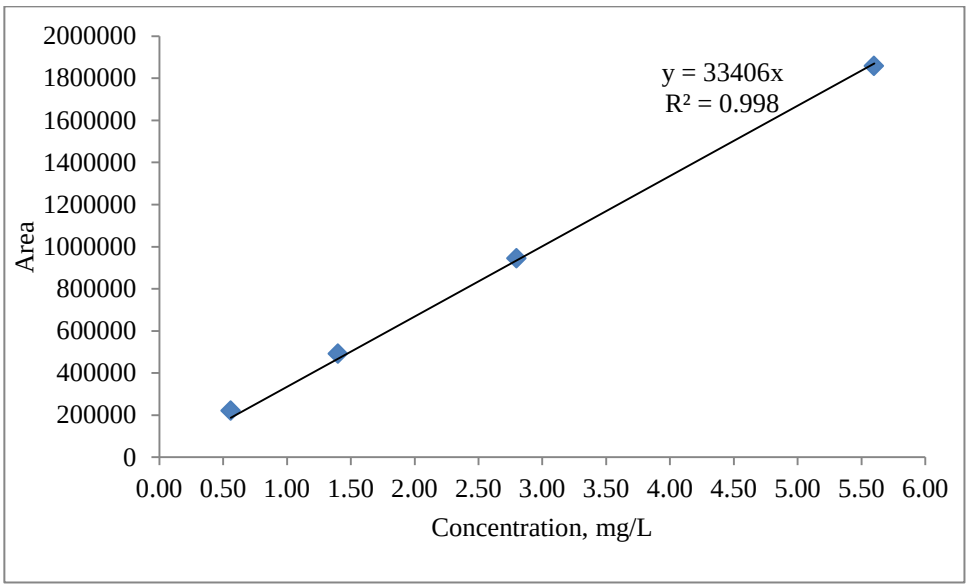

Figure B2: Calibration curve for nitrite-nitrogen standards obtained by HPLC

APPENDIX C

ISI- INDEXED RELATED JOURNAL PUBLICATIONS

1. Mook, W.T., Chakrabarti, M.H., Aroua, M.K., Khan, G.M.A., Ali, B.S., Islam, M.S., Abu Hassan, M.A., 2012. Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review. Desalination 285, 1-13.

2. Mook, W.T., Aroua, M.K.T., Chakrabarti, M.H., Noor, I.M., Irfan, M.F., Low, C.T.J., 2013. A review on the effect of bio-electrodes on denitrification and organic matter removal processes in bio-electrochemical systems. Journal of Industrial and Engineering Chemistry 19, 1-13.

3. Mook, W.T., Aroua, M.K., Chakrabarti, M.H., Low, C.T.J., Aravind, P.V., Brandon, N.P., 2013. The application of nano-crystalline PbO_2 as an anode for the simultaneous bio-electrochemical denitrification and organic matter removal in an up-flow undivided reactor. Electrochimica Acta 94, 327-335.

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Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review

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abstract

Protein rich wastes from aquaculture systems result in total ammonia nitrogen (TAN), total organic carbon (TOC) and biochemical oxygen demand (BOD). A number of conventional approaches have been adopted for the removal of these wastes in aquaculture ponds and hatcheries with varying degrees of success but they face critical problems such as membrane fouling, high cost or the generation of toxic by-products. To overcome such issues, electrochemical technology is commonly employed. The advantages of electrochemical treatment include high efficiency, ambient operating conditions, small equipment sizes, minimal sludge generation and rapid start-up. An even better system involves bio-electrochemical reactors (BERs), which have the potential to generate energy from wastewater (by means of microbial fuel cells) or a valuable product such as hydrogen (using microbial electrolysis cells). Mechanisms of cathodic nitrate reduction and anodic oxidation in electrochemical and bio-electrochemical technology are reported in this review. Also some work on the simultaneous removal of nitrate and organic matter by Electro-Fenton and microbial fuel cells are elaborated upon. It is apparent that BERs can remove contaminants at high efficiencies (≈99%) whilst giving least impact upon the environment.

Contents

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Review

A review on the effect of bio-electrodes on denitrification and organic matter removal processes in bio-electrochemical systems

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ABSTRACT

The main factor that determines the success of a bio-electrochemical system (BES) is the bio-electrode. This paper reviews the direct as well as mediated electron transfer mechanisms in bio-electrodes. Some discussions on their influence upon the performance of microbial fuel and electrolysis cells are considered. Factors affecting organic matter removal at bioanodes and denitrification at biocathodes are elaborated upon. Important parameters for the successful simultaneous removal of contaminants are reported. The major conclusion from this work is that BES is able to remove organic matter and nitrates simultaneously from different wastewater samples at efficiencies greater than 90%.

Contents

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The application of nano-crystalline PbO_2 as an anode for the simultaneous bio-electrochemical denitrification and organic matter removal in an up-flow undivided reactor

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abstract

A nano-crystalline PbO_2 coated carbon composite has been applied as an anode for an up-flow undivided bio-electrochemical reactor (UBER). This electrode provides an enhanced destruction of organic matter in synthetic wastewater in comparison to other anodic materials such as stainless steel, graphite and carbon felts or titanium. The cathode is a granular activated carbon coated with a film of autohydrogenotrophic bacteria. Denitrification occurs simultaneously at the cathode while organic matter is oxidized at the anode. Optimum conditions for the simultaneous removal of organic matter and nitrate from response surface methodology (RSM) studies are an inter-electrode spacing of 3.2 cm, electric current of 18 mA and HRT of 45 h that gave organic matter removal efficiencies of 83% along with 99% removal of nitrate. Further studies on the mechanisms of denitrification and organic matter removal are envisaged.

1. Introduction

Nitrates and organic matter are seriously hazardous to health. For instance, it has been reported that high nitrate concentration in drinking water causes methaemoglobinemia and gastric cancer. Methaemoglobinemia is also known as the blue baby syndrome and occurs normally in infants of ages 0–3 months because they have little methaemoglobin reductase enzymes. When nitrate enters human intestines, it is converted into nitrite that reacts with haemoglobin to form high amounts of methaemoglobin. Since methaemoglobin are non-oxygen carrying compounds, the infant's tissue and organs may lack oxygen that could result in death [1–3]. Similarly an excessive amount of organic matter in drinking water is toxic and can result in the loss of lives [4,5]. Thus, nitrate and organic matter removal from wastewaters is essential before the treated water is discharged to the environment. Maximum emission levels are often set by administrative bodies and an example is the Urban Wastewater Treatment Directive (UWTD) setting EU requirements of 10–15 mg L^{-1} nitrate-nitrogen (depending upon the flow rates), Biochemical Oxygen Demand (BOD) of 25 mg L^{-1} O_2 and Chemical Oxygen Demand (COD) of 125 mg L^{-1} O_2 [6].

The conventional physicochemical treatment processes for organic matter removal include coagulation, flocculation, adsorption, oxidation and membrane treatment [7–11]. However, these processes are expensive, and face critical problems such as the requirement of constant regeneration, membrane fouling, or the generation of toxic by-products. Limitations with such processes and hence the need of alternatives are described in detail in the literature [7]. Similarly, processes such as ion exchange, reverse osmosis and electrodialysis can only eliminate nitrate successfully by decreasing the contaminant from feed to brine thereby requiring further processes to treat the concentrated waste brine prior to discharge to the environment [12]. Many new processes being developed are contaminant specific and do not remove both organic matter and nitrate in one step [13]. In this regard, biological treatment of nitrate becomes most attractive as it converts nitrate into harmless nitrogen gas [14] while organic matter can simultaneously be oxidized to carbon dioxide gas. The use of a bio-electrochemical reactor (BER) has been found to enhance

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